Invertebrate life-history trade-offs and dispersal across a pond-permanence gradient

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Contents

Abstract

Flexible life-history traits and dispersal may allow generalist populations to persist across a range of habitats despite experiencing contrasting selection pressures. Invertebrates exploiting temporary ponds must develop quickly and disperse as adults, or have wide environmental tolerances. Conversely, permanent-pond invertebrates must avoid a suite of predators (e.g., fish and dragonflies). This gradient of pond permanence can result in life-history trade-offs that influence fitness, population dynamics, and genetic structure. In addition, recruitment between habitats may balance juvenile life-history trade-offs and be crucial to sustain generalist invertebrate populations in ponds with unpredictable hydrology. Through a multi-year survey of three pond complexes in the Canterbury high-country and a series of mesocosm experiments using two generalist pond invertebrates, *Xanthocnemis zealandica* damselflies and *Sigara arguta* waterboatmen, I found these two species had alternative life-history strategies that influenced their distributions across the pond-permanence gradient. With longer juvenile development, *X. zealandica* benefited from flexible life-history traits: temporary-pond *X. zealandica* had accelerated development and short-term desiccation tolerance, but were excluded from ponds with long dry periods, whereas, permanent-pond *X. zealandica* had extended development and predator avoidance behaviours (e.g., reduced movement and refuge-use). In contrast, *S. arguta* had an opportunistic life-history strategy with a fixed, rapid development response that allowed them to inhabit more temporary ponds, but they were intolerant of drying and limited to permanent ponds that contained shallow refuges from fish. These results illustrate how alternative life-history strategies enabled two generalist species to achieve broad realised niches.

Recruitment between habitats also appeared to be important for balancing trade-offs and maintaining meta-populations across the pond-permanence gradient. To evaluate the importance of *X. zealandica* dispersal among and within pond complexes I used microsatellite analyses. While there was unique genetic population structure between the North and South Islands, at lower spatial scales there was little variability in genetic diversity and limited genetic structure in populations, likely due to gene flow among different habitat types. Overall, this work shows how an interaction of juvenile strategies and adult dispersal could reduce life-history trade-offs, resulting in weak selection pressures across an unpredictable disturbance gradient. Whether increasingly unpredictable hydrological patterns under climate-warming favour generalist species will likely depend on how well generalist life-history traits and dispersal allow exploitation of a range of habitat types and resilience to variable selection pressures. Higher mean summer rainfall in New Zealand may allow both species to exploit more temporary ponds, whereas longer dry periods between extreme precipitation events could limit *X. zealandica* distributions. Thus, species with generalist strategies are likely to be favoured under warming, but their specific life-history strategies will likely promote or limit their ability to exploit more unpredictable habitats.

Chapter One:

General Introduction

Global changes such as increased climate variability, invasive species spread, and anthropogenic disturbances are driving community homogenisation, favouring generalist species (Clavel et al. 2011, Le Viol 2012, Ponge 2013). These species typically have broad distributions across a range of environments or an ability to consume a variety of food resources, making them more resilient to disturbances than species with more specialised traits (Lurgi et al. 2012, Nagelkerke and Menken 2013). Although generalist species can persist across a wide range of environments with contrasting selection pressures, global change is also causing increased environmental uncertainty due to increased frequency and amplitude of habitat disturbances (Rosenzweig et al. 2008). How populations and communities respond to environmental uncertainty may depend on whether they experience variations in disturbance frequency, duration, predictability, rate of change, or magnitude (Poff and Ward 1989, Poff et al. 1997). These aspects of habitat disturbances can influence the interaction of abiotic and biotic stressors. In particular, the increasing magnitude and frequency of habitat disturbances might decrease the importance of biotic interactions (Poff and Ward 1989, Wellborn et al. 1996). However, the resilience of predators to frequent disturbances can lead to interactive effects of predation and habitat disturbances (Chesson and Huntly 1997, Effenberger et al. 2011, Hermann et al. 2012). Generalist species often have life-history strategies which can mediate abiotic and biotic selection pressures, but unpredictable disturbances that are of higher magnitude or higher frequency could lead to more genetic divergence favouring more specialised traits (Bourne et al. 2014).

Global changes have already led to extirpation of generalist species from a variety of freshwater habitats (Suhonen et al. 2014). Therefore, it is important to understand how different generalist life-history strategies balance the costs and benefits of exploiting unpredictable habitats with variable biotic and abiotic stressors, which could promote or undermine their resilience to further environmental disturbance. Thus, in this thesis I investigated how populations of two generalist invertebrates, *Xanthocnemis zealandica* damselflies and *Sigara arguta* waterboatmen, balanced contrasting selection pressures across a unpredictable pond-permanence gradient through alternative life-history strategies and widespread dispersal. The advantages and constraints of these generalist life-history strategies will elucidate how species can persist across unpredictable disturbance gradients and help predict how susceptible these strategies might be to further uncertainty due to ongoing global changes.

Influences of environmental gradients on species resilience and evolution

The predictability of ecological conditions can influence the evolution and persistence of traits that maximise an organism's fitness in habitats with different selection pressures (Van Tienderen 1991, McPeek 1996). Species with narrow niches are more likely to evolve under predictable selection pressures with adaptations to better exploit and compete for resources, tolerate predictable disturbances, or have specialised predator-avoidance strategies (McPeek 2004, Richter-Boix et al. 2007). However, less stable or unpredictable environmental conditions with unreliable environmental cues likely discourage evolution of traits for specific habitat conditions, and instead favour organisms with broad niches (Van Tienderen 1991, Lytle and Poff 2004, McCauley 2007). However, the mechanisms that permit generalist

species to sustain populations across unpredictable disturbance gradients remains poorly understood.

Generalist organisms which occupy a range of environments across disturbance gradients might rely on a variety of life-history strategies to adapt to, tolerate, or avoid local selection pressures (Verberk et al. 2008a). These life-history strategies include plastic developmental rates, flexible predator-avoidance behaviours, environmentally induced morphologies, opportunistic resource acquisition, or extensive dispersal (Nylin and Gotthard 1998, Altwegg 2002, Lind and Johansson 2007, McCauley 2007). However, these strategies are not without costs (DeWitt et al. 1998, Relyea 2002). To avoid disturbances these organisms might be forced to prioritise juvenile development over adult fitness (e.g., reproductive potential, dispersal capability; Abrams et al. 1996, Rudolf and Rodel 2007, Benard and McCauley 2008). Additionally, unpredictable habitat conditions could result in unreliable cues for critical life-cycle transitions or induce maladapted phenotypes (DeWitt et al. 1998). Therefore, strategies to exploit a range of habitats across environmental disturbance gradients could result in life-history trade-offs, thereby influencing individual fitness, local population dynamics, and regional distributions.

Life-history trade-offs across environmental gradients

Life-history trade-offs can arise from contrasting abiotic and biotic selection pressures across environmental gradients of variable predictability (Lytle et al. 2002). How organisms maximise fitness in unpredictable temporary habitats, despite uncertain time constraints might depend on their developmental flexibility, along with the plasticity of their morphology or physiology (McPeek 1995, Abrams et al. 1996, Zera and Denno 1997, Stoks and McPeek 2003, Suhling et al. 2005). When confronted with deteriorating habitat conditions these

organisms might accelerate growth and development to achieve a life-history stage that can withstand periods of disturbance (e.g., adult diapause, large storage roots, egg aestivation) or allow them to disperse to more favourable habitats (Zera and Denno 1997, Wissinger 1999, Lytle 2001, Suhling et al. 2005, Jannot 2009). These flexibilities might also allow organisms to exploit temporary habitats potentially rich in resources, and with fewer predators and competitors (Kneitel and Chase 2004). However, by maximising survival in temporary habitats they may sacrifice adult fitness and fecundity (Verberk et al. 2008a, Jannot 2009). Moreover, maintaining juvenile development flexible enough to produce different phenotypes, and maintaining mechanisms for gathering and processing environmental information may be costly and limit fitness (DeWitt et al. 1998, Relyea 2002, Lind and Johansson 2009).

In contrast to disturbed habitats, when abiotic selection pressures are reduced due to greater environmental stability, selection from predation and competition might favour alternative life-history traits. To evade predation pressure, organisms might have more cautious behaviour, alter morphology (e.g. spine growth or camouflage) or shift to predator-free subhabitats (Weisser et al. 1999, Johansson 2002, McPeek and Peckarsky 1998, Altwegg 2002, Hoverman and Relyea 2007). These predator-avoidance strategies could also reduce foraging success through lower food encounter rates, diversion of time and energy which could be used for growth and development, or force use of suboptimal refuge habitats with lower food availability and quality (Pierce 1988, Peckarsky 1996, Grabowski and Kimbro 2005). These non-lethal effects of predation can often result in reduced growth and/or slower development (Abrams and Rowe 1996). Thus, there are typically trade-offs associated with biotic selection pressures. Stable environments might permit extended development periods leading to potentially increased adult fitness (e.g., size and fecundity), yet if organisms do not have adequate predator avoidance strategies they might suffer high mortality (Peckarsky

1996, Johansson et al. 2001). Therefore, for organisms to persist across an unpredictable disturbance gradient they need to have a life-history strategy that can minimise their risk to contrasting local abiotic and biotic selection pressure.

Role of dispersal across heterogeneous environments

To balance life-history fitness trade-offs across a range of environments, organisms might disperse between habitats with different abiotic and biotic stresses. Selection may favour dispersal in patchy environments where there are uneven fluctuations in habitat quality between patches (Davis 1986, Benard and McCauley 2008). Under these conditions, widely dispersing organisms could increase their fitness (e.g., offspring success) by colonising higher quality habitats (Den Boer 1990, Hoehn et al. 2007, Benard and McCauley 2008). This exchange of individuals between habitats of variable quality can result in metapopulation dynamics (Hanski 1998). Although metapopulation dynamics might sustain regional populations, these same processes might limit adaptations and speciation across heterogeneous environments thereby promoting generalist traits (Blanquart et al. 2012).

Metapopulation dynamics involving dispersal between different environments might also counterbalance life-history trade-offs. One mechanism could be through bet-hedging behaviour that optimises rather than maximises reproductive efforts across variable habitats by depositing offspring in multiple locations (Hopper 1999, Kisdi 2002, Crean and Marshall 2009, Olofsson et al. 2009). Alternatively, local ecological conditions could cause source-sink population dynamics (Denno and Roderick 1992, Cronin 2003). By dispersing to new habitats, organisms may avoid inbreeding, parasitism, and intra- or inter-specific competition (Doligez and Part 2008). Additionally, more isolated habitats might contain disproportionately fewer predators relative to prey, creating a partial spatial refugium from

predation (Shulman and Chase 2007). However, dispersal is not without costs. As organisms disperse they might waste energy and breeding time searching for new habitats, be vulnerable to predation, and risk being maladapted to conditions of the new habitat (McCauley et al. 2008, Doligez and Part 2008, Bonte et al. 2012). Nevertheless, for organisms dispersing from low-quality habitats with strong selective pressures, the fitness costs of remaining in their natal habitats may be relatively high compared to the costs of dispersal (Moran 1992, Benard and McCauley 2008).

Dispersal may permit organisms to sustain wide distributions across multiple environments despite variable local selection pressures, but the extent of dispersal across different environments can have potential evolutionary consequences. Broad realised niches and dispersal capability are usually positively related (Holt and Gomulkiewicz 1997). Organisms with broad distributions can be more likely to have a higher propensity to disperse between habitats than specialised species (Bonte et al. 2004, Hoehn et al. 2007). These patterns are common across multiple systems and can lead to low genetic differentiation among populations of strong dispersers due to the extensive exchange of individuals, or gene flow, across various environments (Seppa and Laurila 1999, De Block et al. 2005, Huey et al. 2011). However, gene flow across heterogeneous environments could also limit adaptation to local conditions by overwhelming locally adapted genotypes with maladapted immigrant genotypes, thereby reducing potential speciation (Garant et al. 2007, North et al. 2010, Blanquart et al. 2012).

Generalist invertebrate strategies in lentic habitats

Mechanisms that allow populations to persist and disperse across a range of habitats with contrasting abiotic and biotic selection pressures are particularly important in freshwater

ecosystems (Wellborn et al. 1996, Bohonak and Jenkins 2003, Verberk et al. 2008a). In temporary habitats, organisms must undergo quick development and disperse to new habitats or have wide environmental tolerances such as desiccation resistance (Wellborn et al. 1996, Williams 1996, Wissinger 1999). In contrast, in more reliable permanent habitats organisms can be exposed to a suite of predators (e.g., fish and invertebrates) that pose different challenges like predator avoidance (McPeek 2004, Richter-Boix et al. 2007). These differences in predation risk and water permanence between different lentic habitats result in life-history trade-offs that can influence population dynamics and lead to species replacements between habitats (Figure 1.1).

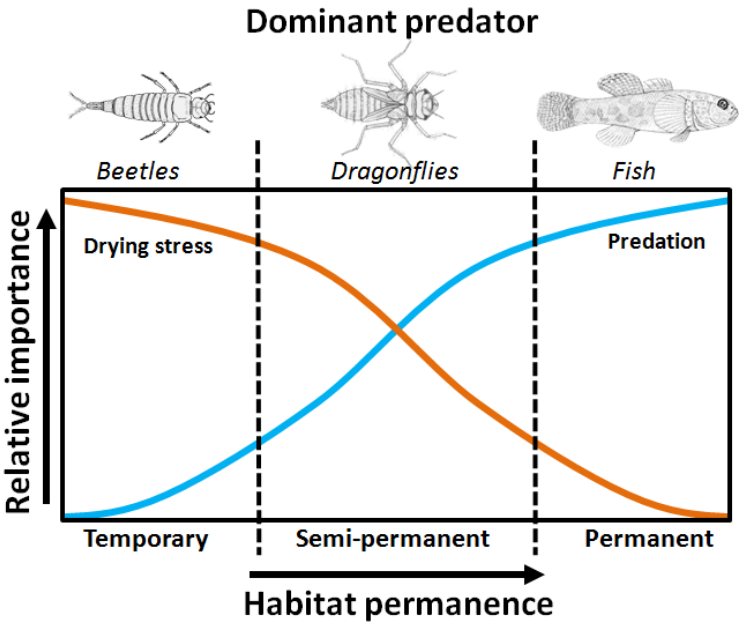


Figure 1.1. Conceptual model illustrating the relative importance of abiotic and biotic selection pressures along the pond-permanence gradient with greater predation risk with increased habitat permanence (modified from Wellborn et al. 1996). Dashed lines indicate pond-permanence categories and their associated dominant predators. This model was developed to explain shifts in invertebrate community structure, but these environmental conditions likely influence life-history trade-offs of species. In particular, to persist across the pond-permanence, gradient generalists would need flexible traits (e.g., developmental, behavioural, or morphological) or avoidance strategies to cope with both drying and fish, whereas specialists could have specific traits that trade-off drying tolerance for predator avoidance or vice-versa.

In North America these species replacements are the result of many aquatic invertebrate specialists having evolved to exploit temporary habitats with predictable seasonal drying, while other species are superior at evading predators in permanent habitats (Wellborn et al. 1996, Wissinger et al. 2003, Lind and Johanssson 2007, Richter-Boix et al. 2007). However, in some places there are some generalist species that can persist across this pond-permanence gradient (McPeek 1996, Van Buskirk 2003, Baber et al. 2004, Werner et al. 2007, McCauley 2008, Wissinger et al. 2009). In particular, there is little evidence for species replacements in New Zealand aquatic invertebrate communities (Winterbourne 1981, Greig 2008, Wissinger et al. 2009). The high proportion of generalist invertebrates across New Zealand lotic and lentic ecosystems has been attributed to unpredictable hydrology, high dispersal between habitats, and weak selection pressures (Winterbourn et al. 1981, Wissinger et al. 2006a, Wissinger et al. 2009, Storey and Quinn 2011, Greig et al. 2013). However, the specific life-history strategies and dispersal dynamics which permit these species to persist across irregularly drying habitats and permanent habitats with predatory fish remain poorly understood.

The presence of generalist invertebrates across an unpredictable pond-permanence gradient may be linked to development and behavioural plasticity (Greig 2008, Wissinger et al. 2009). These flexibilities could allow generalist aquatic invertebrates to take advantage of habitats where water and predator presence are unpredictable or wide-ranging. In addition, traits that confer resilience such as desiccation tolerance could be particularly important in unpredictable temporary ponds. Recruitment between permanent and temporary ponds through adult terrestrial dispersal may also be critical to sustain populations in inter- and intra-annually unpredictable ponds. In particularly dry years temporary ponds may act as population sinks with subsequent colonisation from permanent habitats. But in wet years, temporary habitats could act as refuges from higher permanent habitat predator stresses and

allow more offspring to contribute to the regional population. However, inter-annual unpredictable boom-bust patterns in temporary-pond populations could constrain the ability of species to evolve adaptations to specific abiotic or biotic stresses. Therefore, a combination of flexible juvenile strategies for local habitat conditions and widespread adult dispersal could counterbalance the generalist life-history trade-offs of exploiting habitats across the pond-permanence gradient faced by generalist pond invertebrates (Figure 1.2). Moreover, the strategies generalist organisms use to persist across environmental gradients might determine their population resilience and evolutionary responses to further unpredictable disturbances under global environmental change. Thus, the hypotheses I investigated revolved around whether generalist invertebrates persist across an unpredictable pond-permanence by balancing contrasting selection pressures through juvenile life-history strategies and widespread adult dispersal.

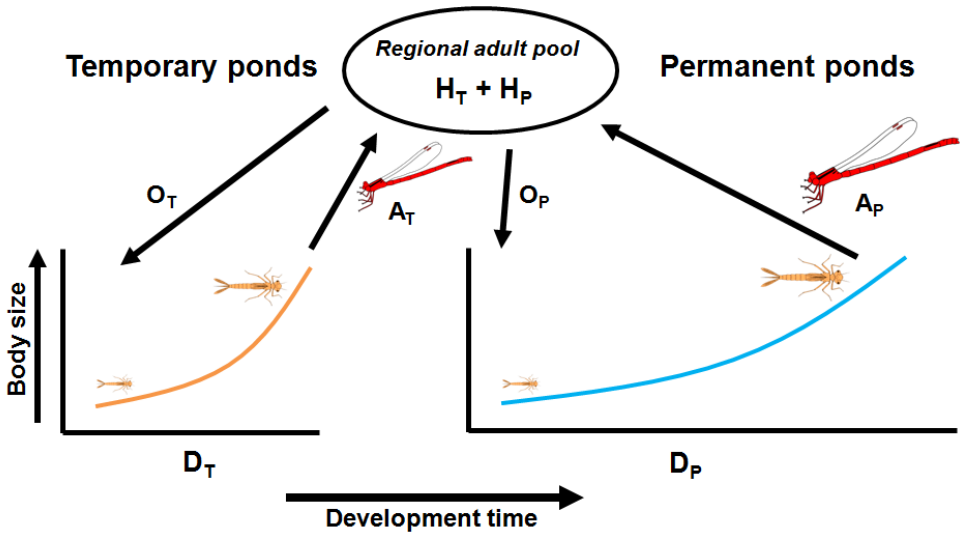


Figure 1.2. A demographic hypothesis based on Peckarsky et al. (2008) illustrating developmental trade-offs between temporary (H_T) and permanent ponds (H_P) and involving bet-hedging recruitment by ovipositing females from the regional adult pool ($O_T = O_P$), and juvenile development influenced by drying stress and behaviour ($D_T < D_P$). The model predicts smaller adults (A_T) emerging from temporary ponds resulting from accelerated development, compared to larger adults (A_P) emerging from permanent ponds with longer development periods.

Thesis layout

This thesis is written as a series of individual papers to be submitted for publication (Chapters Two – Four); therefore, the chapters share some introductory material. Additionally, there is a concluding chapter which amalgamates the three data chapters and includes an evaluation of how global changes may promote or compromise habitat generalist species distributions.

In Chapter Two, using a multi-year field survey, I investigated generalist invertebrate life-history strategies across a pond-permanence gradient in pond complexes throughout New Zealand's South Island high-country. I measured *in situ* juvenile development of two common pond generalists, *Xanthocnemis zealandica* damselflies and *Sigara arguta* waterboatmen, to determine their phenologies and identify any differences in generalist life-history strategies. The constraints associated with these life-history strategies revealed how generalist invertebrates balanced the potential risks of desiccation in inter- and intra-annual unpredictable ponds, and also avoided predators in more permanent ponds.

From the field surveys described in Chapter Two, I showed pond generalists can use flexible or inflexible developmental strategies to persist across the permanence gradient and might have corresponding abilities to avoid, or tolerate, predators and drying. To further understand how the different generalist life-history traits might mediate or limit their distributions across the pond-permanence gradient, I conducted a series of mesocosm experiments in Chapter Three. In these experiments I tested whether the generalist invertebrates had flexible adaptations to natal habitat selection pressures; i.e., predator avoidance behaviours in permanent ponds or desiccation-tolerance in temporary ponds.

Juvenile traits such as development, behaviour, and environmental tolerance might permit generalist invertebrates to exploit a range of pond habitats, adult dispersal between habitats could be critical for generalist invertebrates to balance juvenile life-history trade-offs across

the pond-permanence gradient. Dispersal among different pond types might result in meta-population dynamics which sustain regional populations with temporary ponds experiencing unpredictable turnover events and subsequent recolonisation from permanent ponds. Therefore, in Chapter Four I tested the role of dispersal between and among pond complexes using *X. zealandica* microsatellite markers to measure genetic diversity, population structuring, and gene flow among and within pond complexes in the North and South Island, New Zealand.

Chapter Five integrates the results of Chapters Two to Four to explain how the combination of juvenile life-history strategies and adult dispersal allow generalist invertebrates to persist across an unpredictable disturbance gradient. These insights provide a potential alternative to existing paradigms proposed to explain species replacements across predator-permanence gradients. This alternative paradigm is likely to apply when selection pressures are unpredictable and may explain how generalist invertebrates dominate New Zealand temporary pond communities (Wellborn et al. 1996, Greig 2008, Wissinger et al. 2009). Understanding the strategies New Zealand pond invertebrates use to sustain broad realised niches, and how unpredictable hydrological patterns influence meta-population dynamics and local adaptation, is important under increasingly unpredictable global changes. Thus, I finish by discussing the potential consequences of increasing temperature and unpredictable precipitation/pond hydrology on different generalist life-history strategies and how these patterns may influence biodiversity, species evolution, and vulnerability to global changes.

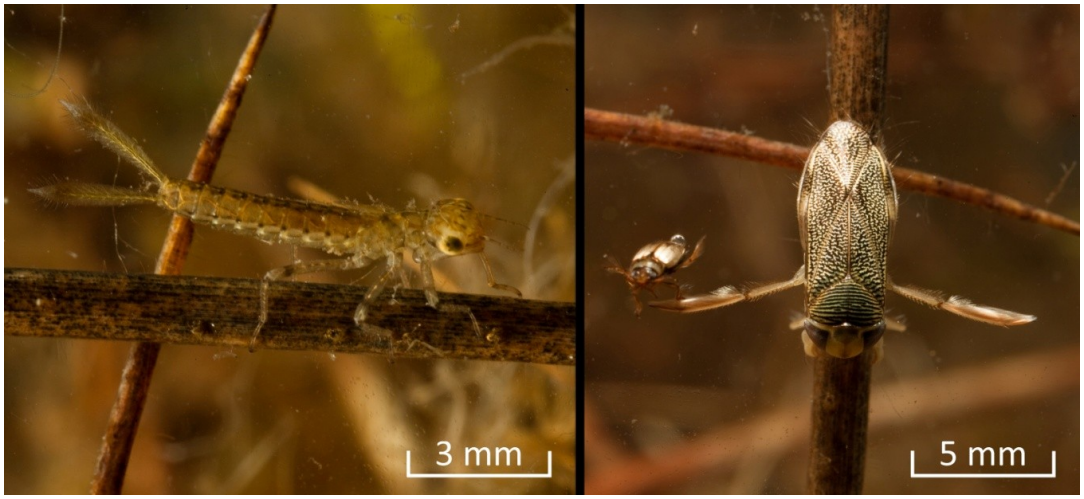


Plate 1. *Xanthocnemis zealandica* damselfly nymph and adult *Sigara arguta* waterboatman are pond generalist invertebrates widespread throughout New Zealand and common across temporary and permanent freshwater ecosystems. Photographs courtesy Angus McIntosh.

Plate 2. Kettlehole ponds and small lakes in the Tarndale area, one of the four pond complexes studied in this thesis. Tarndale is located on the Molesworth Station, Nelson, New Zealand. Photographed in summer 2012.

Plate 3. Time-series showing the dynamic hydrology in a typical temporary pond, Kettlehole, from late spring 2013 to summer 2014 at University of Canterbury's Cass Field Station, Canterbury, New Zealand. Photographs courtesy Sophie Hunt and Amanda Klemmer.

Plate 4. Contrasting environmental selection pressures generalist aquatic invertebrates encounter across New Zealand's unpredictable pond-permanence gradient. Firstly (left), unpredictable temporary-pond drying resulted in many stranded aquatic invertebrates unable to escape desiccation. In contrast (right), predictable semi-permanent and permanent ponds can support predatory fish and invertebrates, like *Procordulia smithii*, pictured here consuming a *Xanthocnemis zealandica* nymph.

Plate 5. Metapopulation dynamics with adult dispersal and bet-hedging behaviours between different ponds are potentially critical to generalist invertebrate persistence across pond-permanence gradients. For example, in February 2012 I observed adult *Xanthocnemis zealandica* ovipositing eggs in neighbouring temporary (left) and semi-permanent ponds (right) within the Tarndale pond complex.

Chapter Two:

Constraints on the flexibility of growth and development control generalist invertebrate distributions across an unpredictable disturbance gradient.

Abstract

Generalists are often able to exploit a range of habitats, but the life-history strategies that allow them to persist across habitats with contrasting selection pressures likely depend on the flexibility of their phenology. Whether habitat generalists are able to vary growth rates and development periods according to local environmental conditions will likely determine how resilient they are to unpredictable disturbances. In a multi-year field survey I investigated *in situ* nymphal growth and developmental strategies of two generalist pond invertebrates, *Xanthocnemis zealandica* damselflies and *Sigara arguta* waterboatmen, across a pond-permanence gradient. In response to habitat drying, I anticipated a flexible generalist response with rapid growth and shortened development to reach maturity before drying, resulting in smaller adult size, whereas I expected nymphs living in ponds with predatory fish would extend growth and development in favour of larger adults. Both generalist species maximised growth rates in temporary ponds but they had different developmental strategies which influenced their distribution. *X. zealandica* had longer development requirements (≥ 135 growth days) which limited their distribution in more unpredictable temporary ponds, whereas *S. arguta* were less constrained in development (≥ 60 days) and inhabited more temporary ponds. The longer development time of *X. zealandica* meant they benefited from flexible life-history traits such as, accelerated development and limited desiccation tolerance

in temporary ponds, and extended development and predator avoidance in permanent ponds. *Sigara arguta* had an opportunistic life-history strategy incorporating a fixed, rapid-developmental response across the permanence-gradient and rapid colonisation of refilled temporary ponds. However, this fixed strategy meant *S. arguta* were intolerant to drying, and were only found in permanent ponds with shallow refuges from fish. Despite their varying development strategies, neither species differed in adult size across the permanence-gradient. Relatively invariable adult size could help sustain generalist populations across the pond permanence-gradient via terrestrial adult dispersal. Overall, my study illustrates how alternative life-history strategies enable generalists to achieve broad distributions in a heterogeneous waterscape, and also highlights how their resilience and flexibility to local selection pressures were dependent on the constraints of their phenologies.

Introduction

The capability of organisms to vary their growth rates can be a mechanism to balance the costs of juvenile mortality and potential benefits of adult fitness (Abrams et al. 1996). By balancing varied growth and development, organisms can have the means to exploit a wide range of environments (Nylin and Gotthard 1998). However, the extent of flexible responses will likely be influenced by the predictability of habitat disturbances. When disturbances are relatively predictable with little temporal heterogeneity, fixed adaptive responses to disturbances are most likely (Lytle 2002). In contrast, in more heterogeneous environments, plasticity is most likely to be favoured (Moran 1992, Thibert-Plante and Hendry 2011). Constraints on the extent of life-history flexibility will also be important in determining the degree of flexibility and ultimately the distribution of a species. In this study I investigated

the flexibility and constraints of life-history strategies affecting two generalist insects that exploit a range of habitats across an unpredictable disturbance gradient.

Being able to persist across spatially and temporally heterogeneous habitats provides the incentives to adapt growth rates and development for periodic habitat availability, season length, variable food resources, or different predation risks (Nylin and Gotthard 1998, Laurila et al. 2002, De Block and Stoks 2005, Jannot 2009). However, when environments are spatially and/or temporally unpredictable organisms are faced with the additional challenge to adapt to volatile conditions (Lytle et al. 2008). By having flexible growth rates and development, species have the potential to exploit a range of unpredictable environmental conditions.

While species with flexible juvenile growth and development might persist in unpredictable environments, there are also inherent fitness costs (DeWitt et al. 1998, Relyea 2002). If growth is accelerated there can be higher physiological demands to complete development, with potential mortality under adverse conditions, or fewer resources allocated to adult size or fecundity (Abrams et al. 1996, Dmitriew and Rowe 2005). Faster growth in animals is often associated with augmented activity and reduced refuge-use which can result in higher predation risk (Abrams et al. 1996, Relyea 2002). Alternatively, if growth rate is reduced to avoid predator detection or to allocate more resources toward adult fitness this can result in longer exposure to predators and a prolonged period of maturation (McPeek and Peckarsky 1998, Relyea 2002). An extended development period might confer greater adult fitness, but an alternative strategy involving faster development with less fecund adults might contribute more generations to regional gene pools (McPeek and Peckarsky 1998, Verberk et al. 2008b). Consequently, it is possible that generalist species inhabiting temporally variable habitats

may adjust their growth rates and development times to local conditions, the limits of their growth flexibility influencing distributions across a habitat disturbance gradient.

In addition to the trade-offs involved in growth and development, there will also be constraints on an organism's ability to counter or adapt to unpredictable local conditions (Moran 1994, Abrams et al. 1996, Lytle et al. 2008). If an organism is unable to complete juvenile development due to deteriorating habitat availability, limited resources, or an inability to evade predators, it can be locally extirpated. Thus, environmental constraints may limit the distributions of generalists attempting to exploit unpredictable habitats (Lytle 2002, McCauley 2008, Greig and Wissinger 2010). Although organisms may suffer high costs due to unpredictable environmental constraints, during favourable periods these same habitats maybe highly rewarding with ample resources, less competition and predation risk. Therefore, there can be advantages to occupying unpredictable habitats. Generalists can have multiple life-history strategies to exploit unpredictable habitats which influence their vulnerability and resilience to different environmental constraints (McCauley 2008, Greig and Wissinger 2010, Verberk et al. 2010).

When generalists exploit a range of habitats they face a challenge to complete development and maximise adult fitness under different environmental constraints (Van Tienderen 1991, De Block and Stoks 2004). To achieve this balance generalists could adopt flexible life-history strategies allowing them to exploit a range of habitats ("jack-of-all-trades") or they could have fixed responses and risk high juvenile mortality in unsuitable habitats ("masters-of-none"). In addition, there may be limits and costs of these generalist life-history strategies. To address these issues I investigated the life-histories of generalist aquatic invertebrates across a pond-permanence gradient using isolated high-country ponds throughout the South Island of New Zealand.

New Zealand lakes and ponds are ideal to carry out such a study because they encompass a range of habitats from permanent shallow lakes containing predatory fish to fishless temporary ponds, with variable inter- and intra-annual hydroperiods due to unpredictable precipitation (Greig 2008). Studies of invertebrate community assemblages of New Zealand streams and ponds have found generalists dominate temporary habitats and are a nested-subset of more diverse communities found more permanent habitats (Winterbourn et al. 1981, Greig 2008, Wissinger et al. 2009, Storey and Quinn 2011).

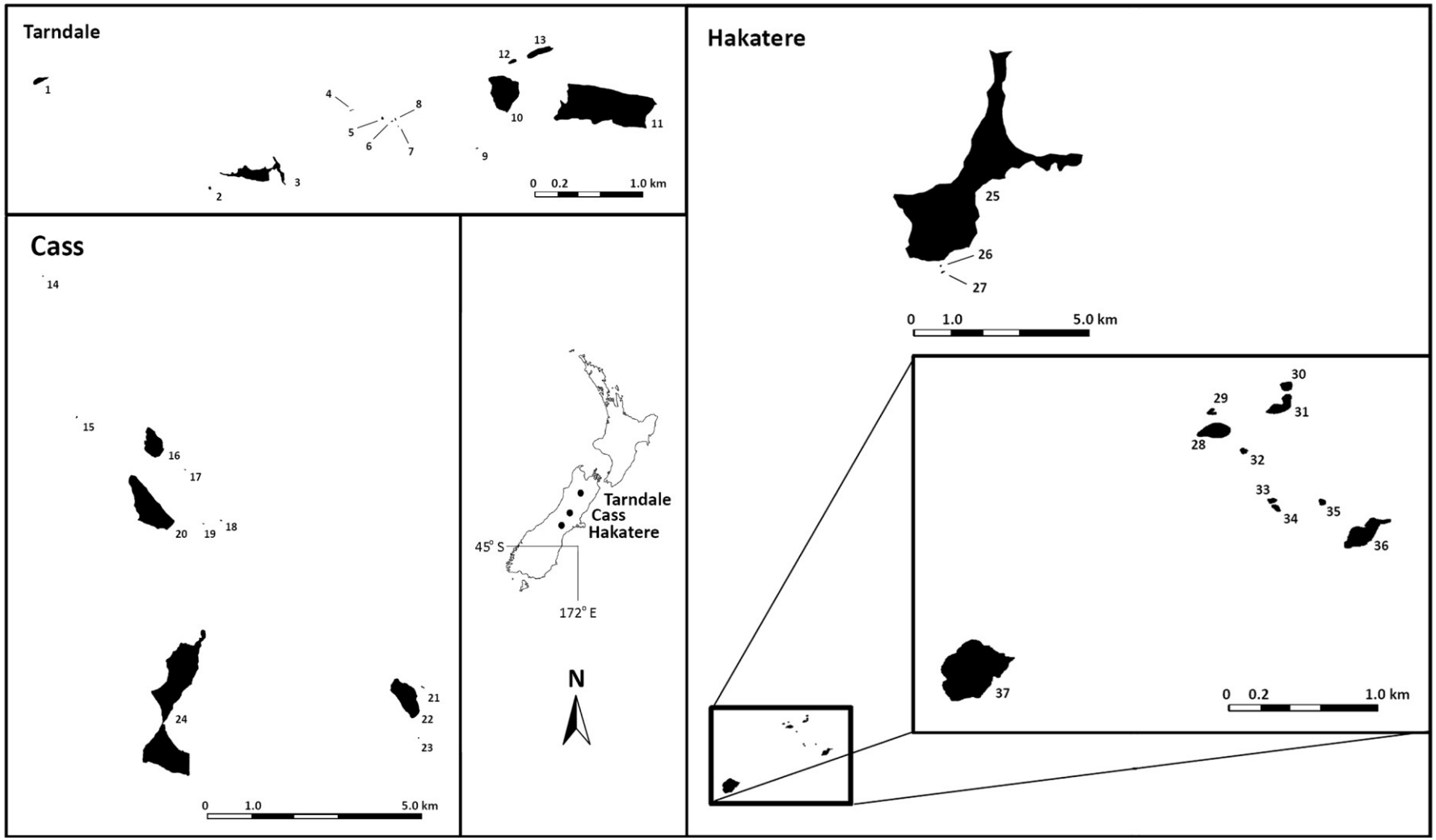
The prevalence of generalist species in temporary freshwater habitats in New Zealand has been attributed to unpredictable precipitation that may limit opportunities for specialisation observed in more predictable North American ecosystems (Wissinger et al. 2009). Using a multi-year field survey of populations across a pond-permanence gradient, I measured *in situ* nymphal development, growth rates, and adult sizes. I predicted that temporary-pond nymphs would have faster growth and therefore shorter development times to avoid inhospitable drying, but with a consequence of reduced adult size. In contrast, I expected the same species in more permanent ponds and lakes would have larger adults as a result of longer, slower development and growth. I also anticipated that there would be constraints to generalist life-history flexibility due to development timing and duration, and limits to their ability to co-exist with different predators.

Methods

Our study was based in the South Island high-country where there are numerous depressional ponds of varying hydrology in a glacially-formed landscape (Figure 2.1). These ponds are commonly situated in complexes comprising permanent lakes, semi-permanent ponds which dry inter-annually, and temporary ponds which usually dry annually in the austral

summer/autumn. The hydrology of these habitats is predominantly influenced by aseasonal rainfall (Figure 2.2), with ponds generally filled during the austral winter (June-September), but having highly variable hydrology during the summer. I surveyed three pond complexes (Tarndale, Cass, and Hakatere) in three catchments (Waimakariri, Clarence, and Ashburton Rivers, respectively) to account for local and regional variation (e.g., precipitation, elevation) that might influence the life-histories of aquatic invertebrates (Figure 2.1). Within each pond complex I chose at least four isolated ponds representing each of three hydrological categories (permanent, semi-permanent, and temporary). In each pond I monitored populations of two insect species, *Xanthocnemis zealandica* damselflies (Odonata) and *Sigara arguta* waterboatmen (Hemiptera), every two months between December 2010 and October 2012, with additional sampling in February and October 2013. These two species were identified as habitat generalists in previous studies of South Island pond community assemblages across gradients of water permanence and predator presence (Greig 2008, Wissinger et al. 2009).

Population monitoring consisted of repeated D-net (1 mm mesh) sweeps of representative littoral and benthic vegetation and substrates to a maximum depth of 2.5 metres. Each sweep included three passes over 1 m x 0.3 m area, with at least five sweeps at each pond. Material collected in sweeps was transferred to sorting trays from which nymphs of the target species were picked out and preserved in 90% ethanol. D-net sweeps were continued until at least 100 nymphs were collected for each species, or material from a total of 20 successive sweeps returned no further nymphs. Identifications were confirmed using standard keys (Rowe 1987, Winterbourn et al. 2006, Young 2010).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0 0.2 1.0 km

Figure 2.1. Three pond complexes in New Zealand's South Island high-country where generalists were collected for *in situ* growth and development analyses. Within each pond complex, individual ponds are indicated by numbers and are associated with different pond types (P = permanent, S = semi-permanent, T = temporary) as indicated below. Tarndale area: 1, Powerline (T); 2, Sedgemere Tarn (S); 3, Lake Sedgemere (P); 4, Roadside (T); 5, 3 Temp 1 (T); 6, 3 Temp 2 (T); 7, 3 Temp 4 (S); 8, 3 Temp 3 (T); 9, Bowscale Tarn (S); 10, Bowscale 2 (P); 11, Bowscale 1; 12, Bowscale 4 (S); 13, Bowscale 3 (P); Cass area: 14, Goldney (S); 15, Rhemus (T); 16, Lake Sarah (P); 17, Kettlehole (T); 18, Bee 2 (S); 19, Bee 1 (T); 20, Lake Grasmere (P); 21, Hawdon Ridge (S); 22, Lake Hawdon (P); 23, HM Depression (T); 24, Lake Pearson (P); Hakatere area: 25, Lake Heron (P); 26, Heron Tarn 1 (T); 27, Heron Tarn 2 (T); 28, Lake Donne (P); 29, Horseshoe (T); 30, Tiny Spider (S); 31, Small Spider (P); 32, Donne Temp (T); 33, Lambies 2 (S); 34, Lambies 1 (T); 35, Fagan Downs Temp (T); 36, Fagan Downs (S); 37, Lake Roundabout (P).

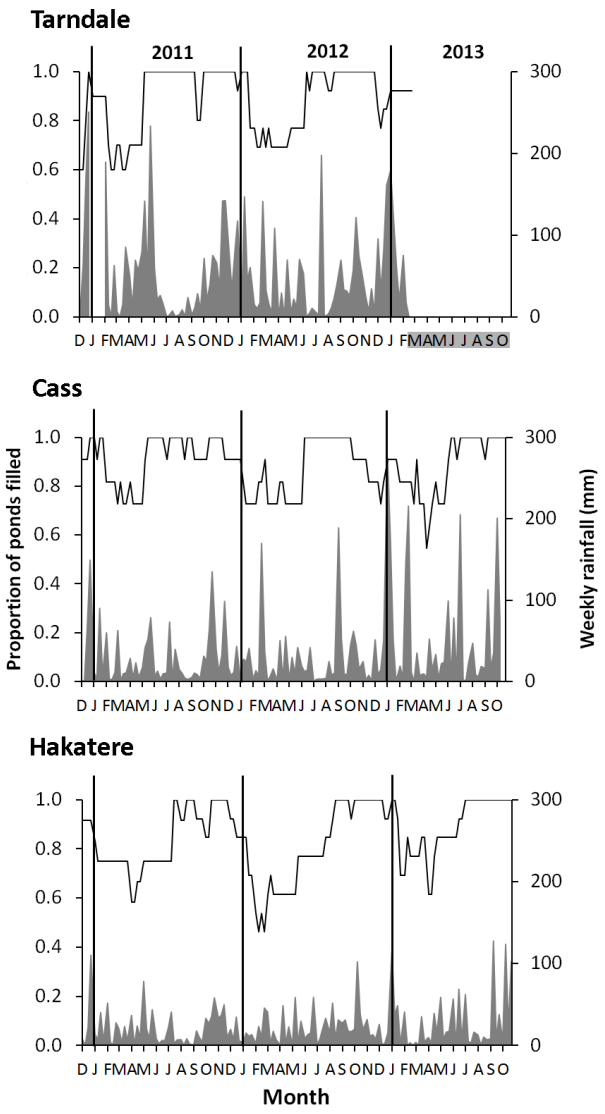

Figure 2.2. The proportion of lakes and ponds with standing water per week between 2010-2013 (black line) in each of the three South Island high-country pond complexes studied. Total weekly rainfall from the nearest rain gauge (<20 km away; Tarndale: NIWA-Mahanga Weather Station [42°1.061'S, 172°38.806'E], Cass: University of Canterbury-Cass Field Station [43°2.07'S, 171°45.579'E], and Hakatere: Environment Canterbury-Boundary Creek Weather Station [43°29.475'S, 170°58.214'E]) over the same time period is shown in dark grey. Water depth and rainfall data were unavailable in the months shaded grey at Tarndale from March to October 2013.

In situ *nymphal development and growth*

To assess *in situ* nymphal development and growth rates in the different pond populations, I measured body lengths of preserved *X. zealandica* and *S. arguta*. Individuals from each pond and sampling event were digitally photographed (>10 megapixels), and body lengths were measured using Adobe Acrobat 7.0 Professional software. Body length measurements were sorted into numerical bins (*X. zealandica* = 1 mm intervals, *S. arguta* = 0.5 mm intervals) and analyzed using the Bhattacharya method of modal progression analysis in FiSAT II v. 1.2.2 (Bhattacharya 1967, Gayanilo et al. 2005). For each pond and sampling period I used the Bhattacharya method to identify the mode of each cohort. These modes were then compiled to measure pond-specific cohort growth over the study period. Growth rates were calculated by converting the initial and final modal cohort body lengths to dry mass (DM) using length-mass regressions; *X. zealandica*: mg DM = $0.0144L^{2.30}$ (Stoffells et al. 2003), and *S. arguta*: mg DM = $0.0098L^{3.05}$ (Greig 2008), where L was length in millimetres. I used DM values to estimate instantaneous growth rates (IGR) from changes in biomass over a given time interval (T); $IGR = \ln(b_2/b_1)/T$, where b_1 and b_2 were the initial and final biomasses, respectively, over the sampling time periods. Adjusted growth rates were also calculated by excluding days when the ponds were dry or below growth thresholds (*X. zealandica:* 8^{o} C [Deacon 1976], *S. arguta*: 10^{o} C [Young 2010]). Due to inter-annual variation in hydrology some temporary ponds did not support nymphal development in part or all of the study period, so I used the growth rates from the three most complete cohorts in my analyses.

Environmental influences on nymphal development

Invertebrate growth is often influenced by temperature, and the timing and duration of pond drying were likely to influence growth and presence/absence of species across a pond-permanence gradient (Nylin and Gotthard 1998, Jannot 2009). Therefore, I monitored water temperature and temporary-pond depth from December 2010-October 2013. Water temperatures were continuously recorded in permanent and semi-permanent ponds with data loggers (Onset HOBO pendant loggers UA-002-64, Bourne, MA USA), while water temperature and depth of temporary ponds were monitored with water-height loggerss (TruTrack HT-100, Christchurch, New Zealand).

Over the course of the field survey *S. arguta* was not found in all the permanent and semi-permanent ponds that had predatory fish. The most common predatory fish in ponds (Table 2.1) were bullies (*Gobiomorphus* spp.), while some also contained koaro (*Galaxias brevipinnis*), longfin eel (*Anguilla dieffenbachia*), brown (*Salmo trutta*), and rainbow (*Oncorhynchus mykiss*) trout (Burrows et al. 1997, Barrier 1998, Wissinger et al. 2006 and 2009). When *S. arguta* nymphs were collected in permanent ponds they were primarily found in shallow littoral zones. Therefore, to determine whether they required shallow littoral refuges, I measured water depth along four transects in all the Cass and Hakatere ponds containing fish. Ten equally-spaced depth measurements were made on each transect which extended from the water margin to a maximum of 1.5 metres deep. The average littoral slope was then calculated for each pond.

Table 2.1. Environmental characteristics and locations of ponds of varying in habitat permanence within three South Island high-country pond complexes that were sampled for *X. zealandica* and *S. arguta in situ* growth. Pond hydroperiod was temporary if the pond dried annually, semi-permanent if it dried inter-annually, or permanent if it consistently contained water. Site names correspond to locations in Figure 1. Elevation, pond area and coordinates were measured with GPS if no published records were available. Fish presence was determined from published records, databases, and confirmed with observations during the field survey.

Complex	Hydroperiod	Site	Elevation (m)	Area (Ha)	Fish	Latitude (S)	Longitude (E)
Tarndale	Permanent	Bowscale 1	1033	25.180	Yes	42°07'41.44"	172°56'58.72"
	Permanent	Lake Sedgemere	1010	12.000	Yes	42°08'08.85"	172°54'54.08"
	Permanent	Bowscale 2	1040	6.910	Yes	42°07'34.77"	172°56'30.30"
	Permanent	Bowscale 3	1055	1.200	Yes	42°07'23.39"	172°56'46.45"
	Semi-permanent	Bowscale 4	1045	0.190	No	42°07'27.74"	172°56'37.63"
	Semi-permanent	Bowscale Tarn	1051	0.014	No	42°07'55.48"	172°56'28.26"
	Semi-permanent	Sedgemere Tarn	1021	0.030	No	42°08'12.26"	172°54'40.81"
	Semi-permanent	3 Temp 4	1060	0.004	No	42°07'50.22"	172°55'53.22"
	Temporary	Roadside	1043	0.030	No	42°07'45.61"	172°55'36.83"
	Temporary	3 Temp 2	1058	0.017	No	42°07'48.53"	172°55'51.02"
	Temporary	3 Temp 1	1053	0.020	No	42°07'47.75"	172°55'47.80"
	Temporary	3 Temp 3	1060	0.010	No	42°07'48.07"	172°55'52.34"
	Temporary	Powerline	1079	0.880	No	42°07'42.71"	172°53'35.02"
Cass	Permanent	Lake Sarah	577	22.290	Yes	43°02'55.28"	171°46'26.04"
	Permanent	Lake Hawdon	579	36.680	Yes	43°06'05.98"	171°51'03.98"
	Permanent	Lake Grasmere	596	65.260	Yes	43°03'57.03"	171°46'24.26"
	Permanent	Lake Pearson	604	194.360	Yes	43°05'29.53"	171°47'04.26"
	Semi-permanent	Hawdon Ridge	636	0.200	No	43°06'06.43"	171°51'15.59"
	Semi-permanent	Goldney	583	0.089	No	43°00'44.69"	171°44'39.25"
	Semi-permanent	Bee 2	641	0.150	No	43°03'56.00"	171°47'43.07"
	Temporary	HM Depression	620	0.070	No	43°06'45.41"	171°51'10.80"
	Temporary	Kettlehole	620	0.090	No	43°03'16.26"	171°47'05.84"
	Temporary	Bee 1	649	0.140	No	43°03'57.96"	171°47'24.09"
	Temporary	Rhemus	592	0.250	No	43°02'34.88"	171°45'12.91"
Hakatere	Permanent	Lake Roundabout	660	11.980	Yes	43°37'19.71"	171°05'51.91"
	Permanent	Lake Donne	662	1.360	Yes	43°36'30.84"	171°06'55.78"
	Permanent	Lake Heron	698	692.550	Yes	43°29'24.04"	171°10'41.80"
	Permanent	Small Spider	665	0.830	No	43°36'26.00"	171°07'15.50"
	Semi-permanent	Fagan Downs	662	2.560	Yes	43°36'54.61"	171°07'42.35"
	Semi-permanent	Tiny Spider	673	0.250	No	43°36'20.67"	171°07'18.29"
	Semi-permanent	Lambies 2	657	0.110	Yes	43°36'45.64"	171°07'14.22"
	Temporary	Donne Temp	659	0.170	No	43°36'34.90"	171°07'05.33"
	Temporary	Fagan Downs Tarn	660	0.070	No	43°36'45.83"	171°07'29.16"
	Temporary	Heron Tarn 1	707	0.120	No	43°29'41.09"	171°10'06.46"
	Temporary	Heron Tarn 2	713	0.320	No	43°29'46.32"	171°10'09.00"
	Temporary	Horseshoe	669	0.100	No	43°36'25.70"	171°06'56.00"
	Temporary	Lambies 1	657	0.090	No	43°36'46.85"	171°07'15.29"

Adult body size

To determine whether there were potential differences in fitness and fecundity between ponds, body sizes of newly emerged adults of both species were measured. Adults were reared from final instar nymphs collected from ponds at Cass and Hakatere in austral spring (November-December 2012; *X. zealandica*) and summer (February-March; *S. arguta*), the peak emergence times of each species. Nymphs (10-20 per chamber) were held in separate emergence chambers (2 L, 150 cm^2 bottom area) for each site and were supplied with: 1.5 L of well water, 2-cm pebble substrate, wood pegs to assist emergence, and fed daily either zooplankton for *X. zealandica* or filamentous algae for *S. arguta*. Most adults emerged within 48 hours, and were collected and frozen at -20^o C. Adult body length was measured using the same digital photography methods as in the field survey. Because egg production is delayed in both species (Rowe 1987, Young 2010), eggs could not be used as a measure of fecundity of newly emerged adults. To avoid influences on adult fitness due to conditions outside natal habitat conditions, I opted not to retain adults in captivity until egg production began or continued to fruition.

Statistical analyses

We evaluated the *in situ* growth rate differences across the pond-permanence gradient using linear mixed effects models (LME). In each model habitat type was the fixed effect, with cohort and pond nested within pond complex as random effects to account for regional and temporal variation (e.g., elevation, inter-annual temperature and precipitation). For each species, separate models were tested with average and adjusted growth rates using lmer from the lme4 (Bates et al. 2013) packages in R version 3.0.2 (R Development Core Team 2013). Where significant fixed

effects were found Tukey post-hoc tests were used to determine where differences lay with the multcomp package (Hothorn et al. 2014). Random effects were assessed using log-likelihood ratio tests in the lmerTest package (Kuznetsova et al. 2013).

To determine the minimum number of days required to complete nymph development, I used a logistic regression of the presence/absence of each species across all ponds based on their annual available growth days averaged over all three years. Annual available growth days were calculated from the number of days each year a pond was filled and the water temperature was above the species' growth threshold. I also tested whether the presence/absence of *S. arguta* was influenced by the shoreline slope in fish ponds, and determined their threshold response using a logistic regression in R.

To test the influence of variable nymphal growth rates on adult body size across the permanence gradient I analyzed adult body lengths using LME. For each species, habitat type and sex were modelled as fixed effects with source pond nested within pond complex to account for potential spatial variation in body size. Significant fixed effects were analyzed using Tukey and log-likelihood ratio tests for random effects.

Results

In situ *growth rates*

The growth rates of both generalist species differed significantly across the pond-permanence gradient (*X. zealandica*: $F_{2,18.7} = 15.32$, $p < 0.001$; *S. arguta*: $F_{2,46} = 7.71$) with higher growth in temporary ponds (*X. zealandica*: $p < 0.001$; *S. arguta*: $p = 0.001$) than in either semi-permanent

or permanent ponds. In addition, growth in semi-permanent ponds was not different to permanent ponds for either *X. zealandica* ($p = 0.06$) or *S. arguta* ($p = 0.34$) (Figure 2.3). There were no significant random effects of pond complex or cohort, but there were source-pond differences for *X. zealandica* ($p = 0.02$), but not *S. arguta* ($p = 0.13$). When growth rates were adjusted to exclude days when ponds were dry and below growth threshold temperatures, differences between pond types increased for both generalist species (*X. zealandica*: $F_{2,22.5} = 26.36$, $p < 0.001$; *S. arguta*: $F_{2,25.8} = 0.001$). Growth in temporary ponds was significantly different (*X. zealandica*: $p < 0.001$, *S. arguta*: $p < 0.001$) from both semi-permanent and permanent ponds (*X. zealandica*: $p = 0.18$, *S. arguta*: $p = 0.74$) (Figure 2.3). Again, the only significant random effect was source-pond (*X. zealandica*: $p < 0.001$, *S. arguta*: $p = 0.048$).

These growth differences were also reflected in the development and life cycles of each species across pond types. Overall, *S. arguta* had much higher growth than *X. zealandica*. *S. arguta* were able to complete at least one generation in each pond type within a summer, with the shortest development times occurring in temporary ponds (Figure 2.4). In contrast, *X. zealandica* in temporary ponds had uni-voltine life-cycles (i.e. single annual generation), whereas in more stable permanent and semi-permanent ponds they had semi-voltine development (i.e. generations requiring multiple years) (Figure 2.4).

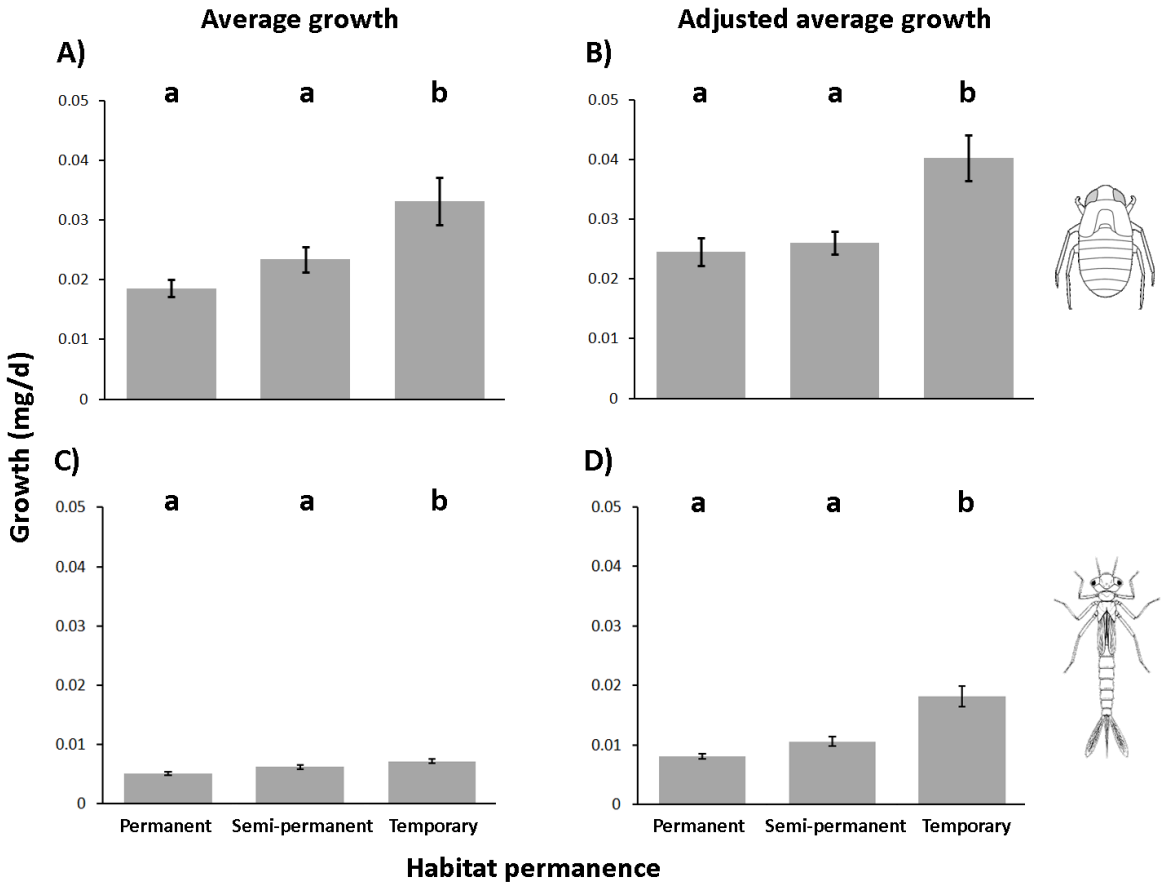

Figure 2.3. Mean (± S.E.) growth rates of *S. arguta* (A and B) and *X. zealandica* (C and D) in three pond-permanence classes. Adjusted values (B and D) were calculated solely from data obtained when ponds contained water and water temperatures were above the minimum threshold for growth (*S. arguta*: threshold temperature, 10°C; *X. zealandica*: threshold temperature, 8°C). Letters above the histogram indicate significant relationships based on post-hoc tests.

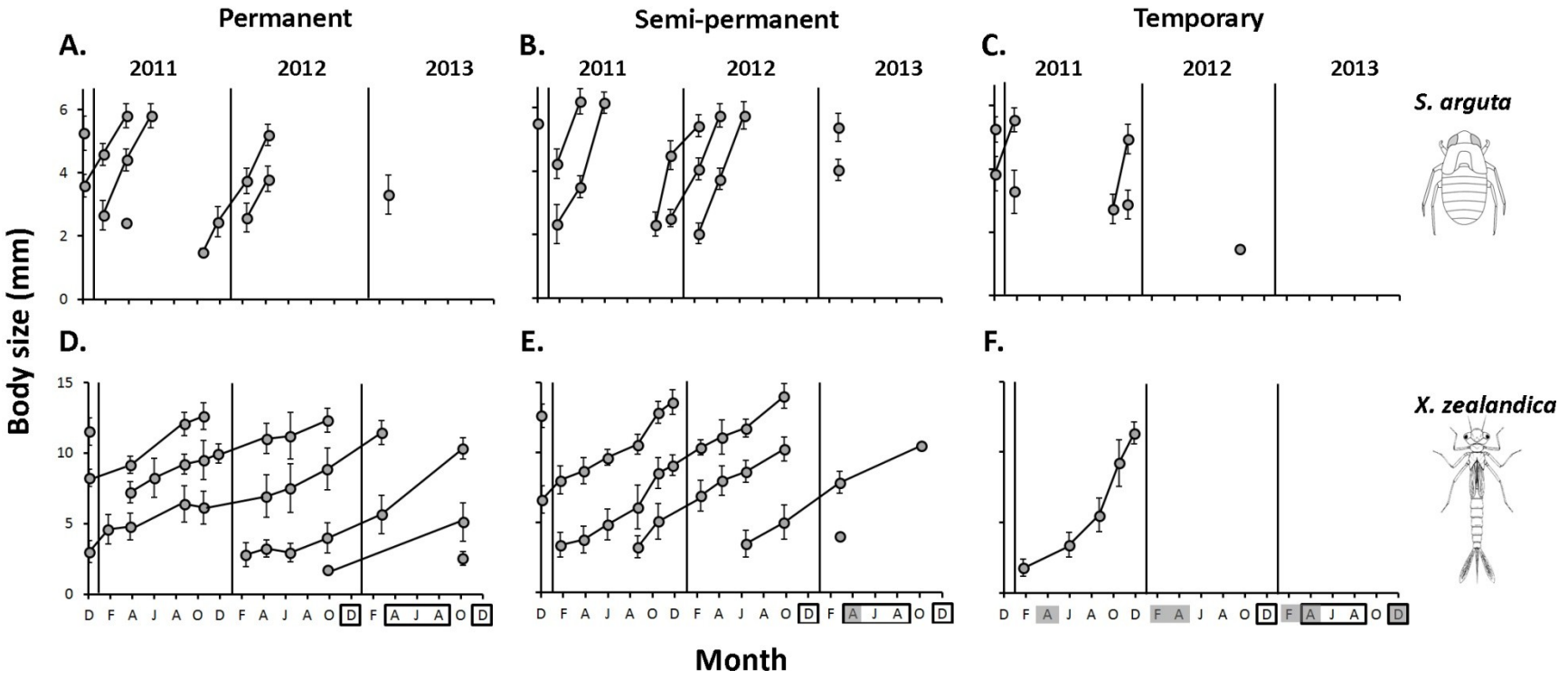

Figure 2.4. Changes in body size (mean ± S.E.) of *S. arguta* (A-C) and *X. zealandica* (D-F) cohorts across a pond-permanence gradient in three Cass ponds in the Canterbury high country (permanent: Lake Sarah, A and D; semi-permanent: Goldney, B and E; and temporary: Kettle, C and F) between December 2010-October 2013. Grey shaded months indicate the ponds were dry during the sampling period and outlined months represent dates when the ponds were not sampled.

Biotic and abiotic influences on nymphal development

There was a negative growth relationship across the pond-permanence gradient with higher growth rates in ponds with fewer available growth days and lower growth in ponds with greater numbers of growth days. Nymph growth rates of both species were highest in temporary ponds (*X. zealandica*: $R^2 = 0.55$, $p < 0.001$; *S. arguta*: $R^2 = 0.54$, $p < 0.001$), but some short-term temporary ponds had an inadequate number of available growth days to sustain nymph development (Figure 2.5). However, the species had different developmental thresholds with *S. arguta* requiring at least 61 growth days ($\chi^2 = 25.3$, $p < 0.001$) compared to 135 growth days for *X. zealandica* ($\chi^2 = 35.9$, $p < 0.001$). Above *X. zealandica*'s developmental threshold they were able to mature across the rest of the permanence gradient. In contrast, *S. arguta* were also excluded from particular permanent ponds with fish (Figure 2.5). In ponds containing fish, *S. arguta* were constrained to those with shallow littoral zones with no greater than a shore slope < 8.36 m/cm depth ($\chi^2 = 13.5$, $p < 0.001$) (Figure 2.6). Therefore, *S. arguta* were only found with predatory fish when shallow refuge habitats were available.

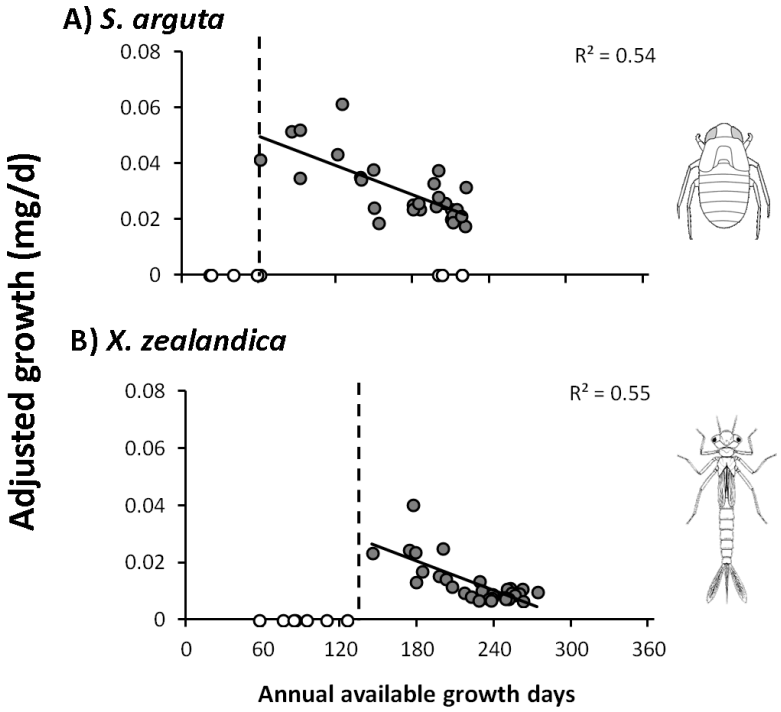

Figure 2.5. Site-specific adjusted growth rates in relation to the average available days for growth for *S. arguta* (A) and *X. zealandica* (B). Available growth days accounted for days above temperature thresholds and when ponds had standing water. Grey data points indicate ponds where growth was sufficient to complete nymphal development. Open points are ponds where early instar nymphs or adult oviposition were found but nymphs were unable to complete development. The dotted line denotes the threshold for the number of available growth days for each species to complete nymphal development (*S. arguta*: 61 days, *X. zealandica*: 135 days).

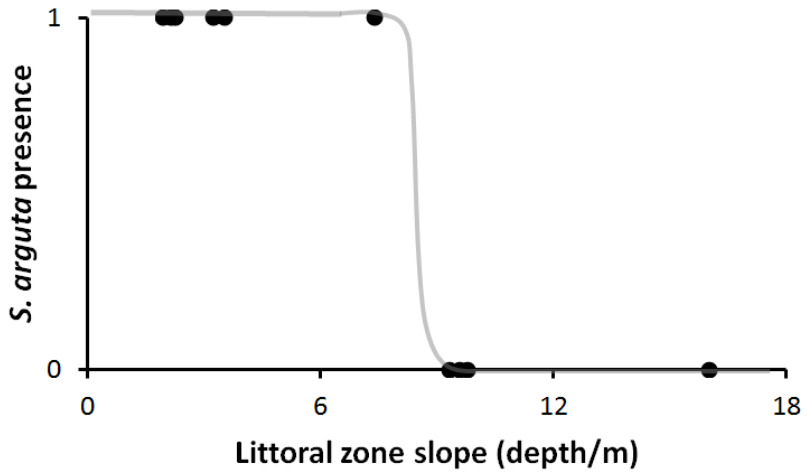

Figure 2.6. Presence (1) and absence (0) of *S. arguta* in relation to the slopes of the littoral zones of Cass and Hakatere permanent and semi-permanent ponds containing fish in the South Island high country. The line indicates the slope threshold-response where *S. arguta* were not present in ponds with steep banks (> 8.3 cm depth/metre).

Adult body size

Neither generalist species had significantly different adult body sizes across the pond permanence-gradient (*X. zealandica*: $F_{2,14.94} = 2.03$, $p = 0.12$; *S. arguta*: $F_{2,9.36} = 0.09$, $p = 0.91$). *S. arguta* females had larger body sizes than males ($p < 0.001$), and *X. zealandica* body size did not differ between sexes ($p = 0.43$) (Figure 2.7). There were no random effects of pond complex, but there was variation in individual body size between ponds (*X. zealandica*: $p < 0.001$, *S. arguta*: $p < 0.001$). Thus, although there were considerable systematic differences in development and growth associated with pond permanence, these did not lead to differences in adult body size.

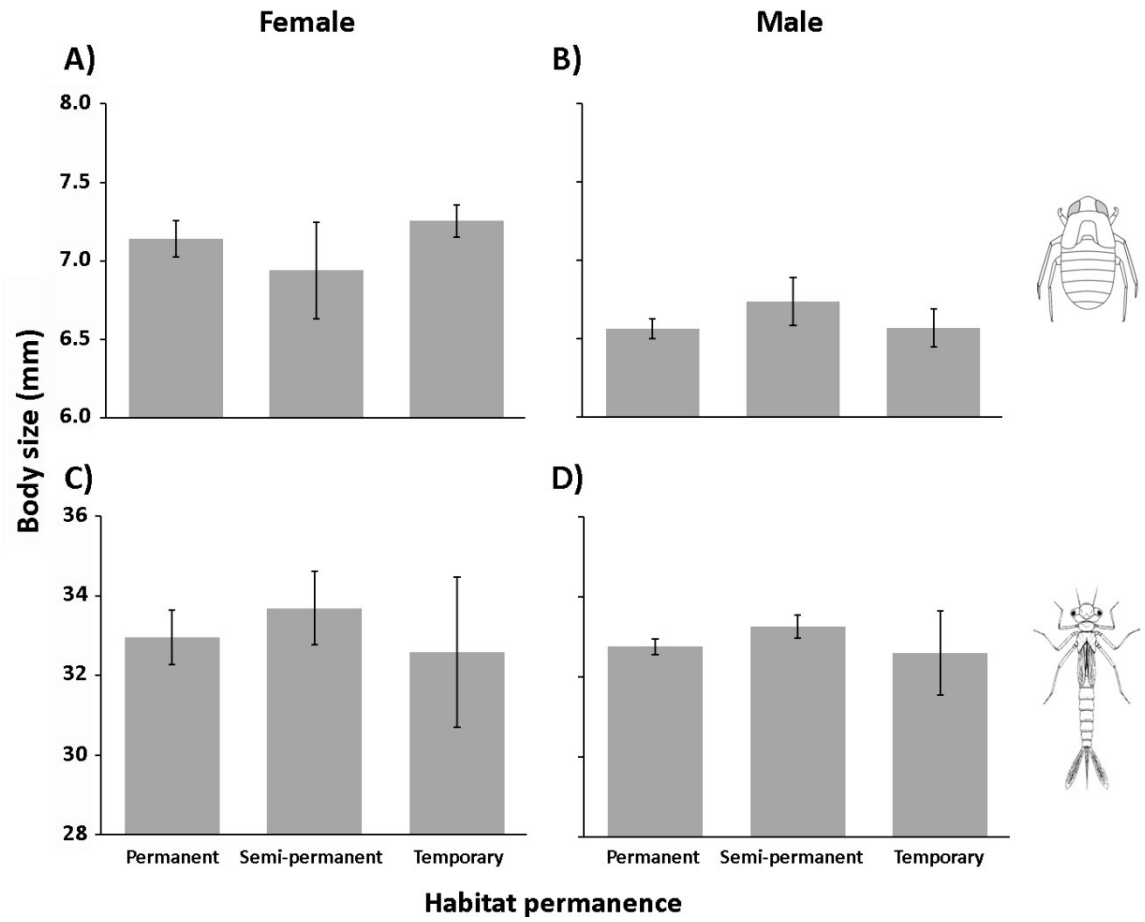

Figure 2.7. Adult body size (mean ± S.E.) of female and male *S. arguta* (A and B) and *X. zealandica* (C and D) for the three pond-permanence classes.

Discussion

The extent that generalists can vary growth rates and development can be linked to the different life-history strategies they use to exploit heterogeneous and unpredictable environments (Nylin and Gotthard 1998, Laurila et al. 2002). However, there will be limits and costs for generalists exploiting these environments, and how well they cope with such constraints will influence their vulnerability and resilience to disturbances. In my study I found two generalist species persisted across dramatic and unpredictable pond permanence-

gradients using different life-history strategies which influenced their growth and development flexibility to local environmental conditions.

Flexible and inflexible life-history strategies have been investigated in the past, but the mechanisms and constraints of these different strategies have not been tested in unpredictable ecosystems (Lima 1998, McPeek 1996, McCauley 2008, Greig and Wissinger 2010). I found both generalist species had faster growth in temporary ponds than in semi-permanent and permanent ponds, but the flexibility of development time differed greatly between species. *X. zealandica* had longer nymphal development and greater differences in the length of development period, and was also able to survive short-term drying and avoid predators in more permanent habitats. In contrast, *S. arguta* was a rapid coloniser, with a shorter, less variable development period that enabled them to exploit temporary ponds before they dried. However, this less flexible strategy likely made them more susceptible to drying mortality and fish predation (Chapter Three).

These different life-history strategies had consequences for each species' ability to exploit unpredictable temporary ponds as well as permanent ponds with predatory fish, restricting their distributions across the pond-permanence gradient. Although *X. zealandica* had a flexible life-history strategy, they were excluded from the more temporary ponds likely due to their longer development, but were not limited in ponds with more predictable hydrology. In contrast, *S. arguta*'s short and inflexible development enabled them to occupy most temporary ponds, although they were absent from some ponds containing fish. In ponds where generalists were able to complete development, both species had consistent adult body sizes across the pond-permanence gradient despite their variable growth rates and development in response to local abiotic and biotic stresses. My results indicate that generalist species can use different life-history strategies to exploit a range of heterogeneous

environments, but their distributions are also constrained by the extent of their flexibility to unpredictable disturbances.

In situ *nymphal development and growth*

I originally predicted that the two species would have flexible life-histories across the pond-permanence gradient, with accelerated growth and development in temporary ponds. In accordance with this prediction I found accelerated growth in temporary ponds by both species. In the more predictable and stable environmental conditions provided by semi-permanent and permanent ponds, both species had slower growth and longer development. These results support models whereby growth rate is adaptively flexible and can be increased under time constraints or other deteriorating environmental conditions (Abrams et al. 1996). Such flexibility is likely to be the cornerstone of a generalist life-history strategy.

While rapid growth is probably an adaptive response to unpredictable habitat availability, proximate disturbance cues can have an important influence on life-history flexibility. However, abrupt, unpredictable, disturbances such as flash-floods or fires can lack environmental cues indicating change and therefore, limit an organisms' ability to adapt to rapidly changing conditions (Lytle 2001 and 2006). Nevertheless, the likelihood of abrupt environmental disturbances can favour inflexible rapid growth and development to avoid experiencing catastrophic disturbances or traits that confer tolerance (Lytle 2001, Bogan et al. 2013). The time that temporary ponds held water was unpredictable in my system, but gradual drying could still provide proximate environmental signals that trigger flexible, rapid growth responses. Accelerated growth and development in organisms experiencing drying conditions has been attributed to more variable temperatures in shallower water, crowding, and changes in salinity and conductivity (Wellborn 1996, Rudolf and Rödel 2007). Under

these deteriorating conditions, juvenile growth may be maximised to achieve a less vulnerable life-stage and avoid high mortality before the habitat completely dries. I found generalists had flexible growth-rate responses to pond drying, but the extent of developmental flexibility was not uniform between species across the pond-permanence gradient.

I found the degree of developmental flexibility was associated with two alternative life-history strategies: slow and flexible, versus fast and inflexible. *X. zealandica* had flexible development across the pond-permanence gradient, with temporary pond populations completing development in less than a year, while semi-permanent and permanent populations took two years. Rapidly developing *S. arguta,* in contrast, were able to complete at least one generation annually across most ponds. Both species increased growth rates to exploit temporary ponds, but their slower development in more stable conditions suggests there were trade-offs linked to co-existing with predators versus tolerating desiccation. Such growth and developmental trade-offs are common and critical to organisms that encounter variable selection pressures across heterogeneous habitats (Stevens et al. 2000, Altwegg 2002, De Block and Stoks 2004, Jannot et al. 2008).

Across multiple ecological systems, plastic responses to heterogeneous environments are limited by environmental cue detection, development costs of alternative phenotypes, developmental time requirements, and limits to behavioural flexibility or abiotic tolerance (DeWitt et al. 1998, Relyea 2002). Furthermore, unreliable, unpredictable and rapidly changing conditions can limit the evolution of adaptive responses (Givnish 2002). Potential costs associated with maximised juvenile growth have been linked to higher energy requirements, increased predator detection, reduced immune responses, smaller adult size, and lower fecundity (Abrams et al. 1996, Peckarsky and McPeek 1996, Stoks et al. 2005a). I

found constraints were linked to life-history strategies, which limited the distribution of the two species along the pond-permanence gradient, depending on their ability to adapt to or avoid pond drying and predatory fish.

Environmental constraints on generalist life-history strategies

Both species were able to exploit habitats across the pond-permanence gradient with flexible growth responses to local environmental conditions, yet neither was ubiquitous across the entire pond-permanence gradient. Instead, the distributions of each generalist species depended on their developmental constraints and the likelihood that they would experience and be able to adapt to local environmental stresses.

Over the course of my study, the timing and duration of temporary pond drying was highly unpredictable and the wetting of each pond varied intra- and inter-annually. Both species were limited by their ability to accelerate growth rates, which constrained their development across these dynamic and unpredictable conditions. However, with their alternative life-history strategies, they had different developmental thresholds and abilities to tolerate drying, which influenced their distributions among temporary ponds. *X. zealandica* nymphs required more time to complete development, but also had evidence of short-term desiccation tolerance which allowed them to persist in temporary ponds with short dry periods (Figure 2.4). However, there were limits to their drying tolerance and *X. zealandica* nymphs were excluded from temporary ponds that remained dry for more than the minimum 135 days required to complete growth. In contrast, if a temporary pond dried, *S. arguta* were unable to withstand desiccation. Despite this potentially high mortality cost, they were able to exploit more temporary ponds due to their ability to colonise rapidly and complete nymph development before ponds dried. These contrasting responses to dry conditions were

illustrated in experiments, where *X. zealandica* survived 16 days without water, while *S. arguta* had no drying tolerance (Chapter Three: Figure 3.4). Even though there are constraints associated with both flexible and inflexible generalist life-history strategies limiting persistence in unpredictable temporary ponds, there is likely to be a combination of traits with sufficient advantages (e.g., higher food resources, fewer competitors, less predation risk) combined with weak inter-annual selection pressures that enable these generalist species to continue to exploit these habitats. Therefore, there may be frequent oscillations between beneficial periods where exploiting unpredictable habitats pays off and periods when mortality costs are high. These environmental oscillations could restrict the evolution of specialised adaptations (Kisdi 2002).

Selection pressure associated with predation is also likely to play a role in determining distributions across heterogeneous habitats and have been observed elsewhere to be a result of their anti-predator behaviours (Altwegg 2002, McCauley 2008, Greig and Wissinger 2010). In more permanent ponds, neither generalist species was constrained by pond duration, but predators likely influenced the presence of each species, depending on their life-history strategy. *X. zealandica* nymphs were present in all permanent ponds, and commonly found throughout the benthos, and on emergent and submergent vegetation. In contrast, *S. arguta* nymphs were not found in permanent ponds (Figure 2.5) with steep littoral zones (Figure 2.6). These distribution patterns were likely linked to anti-predator behaviours, with *X. zealandica* possibly having better predator avoidance behaviours, albeit with slower growth and development. In contrast, *S. arguta* appear to lack flexible predator-avoidance behaviours, constraining them to permanent lakes with refuge sub-habitats. Behavioural differences between these generalists through behavioural observations demonstrated permanent-pond *X. zealandica* were less active and spent more time in refuges than temporary-pond *X. zealandica* or *S. arguta* when exposed to caged fish, although both species

experienced high mortality when exposed to fish in mesocosms lacking habitat complexity (Chapter Three). Combined with my results here, these observations suggest fish likely exclude *S. arguta* from permanent ponds lacking refugia and *X. zealandica* readily coexist with predatory fish in habitats with natural habitat complexity. *X. zealandica*'s ability to moderately adjust anti-predator behaviours according to their natal habitat conditions mirrors other studies reporting generalists have moderate growth and behavioural responses to predator cues compared to specialists (Caley et al. 2003, McCauley 2008). However, these imperfect strategies to coexist with fish suggests there are limits to generalist anti-predator flexibility, as I found with permanent pond *X. zealandica*. These results suggest the distributional patterns of generalist species in more predictable habitats can be linked to their ability to flexibly respond to predator selection pressures.

This developmentally- and behaviourally-flexible strategy contrasts with other studies that found generalists with fixed life-history strategies can evolve if there are convergent selection pressures across disturbance gradients (McPeek 1996, Greig and Wissinger 2010). For example, Greig and Wissinger (2010) found a fixed-trait response in a caddisfly, *Asynarchus nigriculus*, across a pond-permanence gradient due to the convergent benefits of rapid development under temporary pond drying and the avoidance of peak predation periods in permanent ponds. While *S. arguta*'s intolerance to drying is a strong motivator for rapid development in temporary ponds, shallow refuges in permanent habitats might also be seasonally limited because lake levels can greatly fluctuate in depth with draw-downs in late summer/autumn. This suggests permanent pond *S. arguta* may also benefit from rapid development to exploit seasonal shallow refuges. Therefore, whether generalists have evolved flexible anti-predator behaviour may depend on whether they can avoid co-existing with predators during their development.

Because the flexible life-history of *X. zealandica* shows evidence of adaptive responses to both predators and drying pressures it is tempting to conclude that they are a "jack-of-all-trades". Nevertheless, their flexibility still had potentially costly limits which prevented them from tolerating long dry periods. In contrast, *S. arguta*'s fast and inflexible strategy, or "master-of-none", was also potentially risky because they could not adjust to local abiotic and biotic stresses. However, this disadvantage was offset by their ability to occupy a greater range of habitats across the pond-permanence gradient. That two generalist species with alternative life-history strategies were able to exploit similar habitats across the pond-permanence gradient despite variable abiotic and biotic selection pressures suggests overall that heterogeneous ponds exert weak selection pressure. Models of variable selection pressures in heterogeneous environments indicate generalists and polymorphisms are usually retained across multiple environments only if organisms experience weak selection pressures, whereas more stable or strong selection leads to the evolution of specialists (de Meeus and Goudet 2000, Debarre and Gandon 2011, Massol 2013).

In my system, weak selection could be the result of aseasonal, unpredictable disturbances, weak predation pressure in permanent ponds, high connectivity between neighbouring habitats, and New Zealand's moderate climate (Wissinger et al. 2006 and 2009, Greig et al. 2013). This situation contrasts with that provided by ecosystems that experience predictable seasonal disturbances allowing species to evolve specialisations to alternative selection pressures along habitat-permanence gradients (Wellborn 1999). Ecosystems with severe and unpredictable regional disturbances (e.g., intermittent desert streams) typically experience harsher environmental conditions, greater developmental time constraints, and more isolated populations, resulting in higher proportions of specialist species (Bogan et al. 2013). Thus, the predictability and severity of disturbances, along with habitat connectivity, might

influence whether species can sustain broad niches with generalist strategies or evolve specialisations for local conditions.

The evolution of alternative generalist life-history strategies that facilitate the exploitation of habitats across a disturbance gradient may depend on the likelihood that individuals will encounter the environmental stress during their life-span. The differences I observed between the life-histories of the two generalist species, and their associated developmental constraints across the pond-permanence gradient, are consistent with various models of organism adaptation to disturbance regimes (Van Tiederen 1991, Lytle 2001, Rudolf and Rödel 2007). In particular, organisms with fast growth and rapid maturity should not respond as strongly to disturbances as slow-growing and long-lived organisms (Lytle 2001). This is largely linked to the idea that disturbances must be frequent enough to have an impact on an organism during its life-span to bring about an evolutionary response (Venable and Brown 1988, Turner et al. 1998). Therefore, opportunistic organisms (e.g., *S. arguta*) that colonise temporary habitats early and complete development before experiencing a disturbance have little evolutionary incentive to adapt to local conditions. Instead these organisms should maintain a rapid growth strategy and early maturity, even if they experience high mortality during disturbances (Lytle 2001). Following this same argument, slower-growing organisms (e.g., *X. zealandica*) should have evolved adaptation to coexist with predatory fish and disturbance regimes, such as short-term desiccation tolerance, as I have found in this study (and see Chapter Three). With longer development periods in unpredictable habitats, these organisms benefit most from a flexible response to disturbance where they can either capitalise on potential fitness benefits of additional growth when habitats persist longer, or accelerate growth under less favourable conditions (Rudolf and Rödel 2007). However, there can still be adaptation limits to more frequent or severe disturbances (Lytle 2001, Lytle et al. 2008). If organisms are able to adapt to deteriorating environmental conditions, and accelerate their juvenile growth to avoid

mortality due to habitat disturbance, there is an expectation that there will be potential repercussions for adult fitness (e.g., smaller size, lower fecundity, limited dispersal capacity) (De Block and Stoks 2005, Dmitriew et al. 2007, Benard and McCauley 2009).

Adult body sizes

Alternative juvenile life-history strategies may allow organisms to occupy habitats with different selection pressures but developmental responses to environmental stresses can result in carry-over effects for adults (Stevens et al. 2000, De Block and Stoks 2005, Dmitriew et al. 2007,Benard and McCauley 2009). Despite having different life-history strategies, *X. zealandica* and *S. arguta* adult size was conserved in both species across the pond-permanence gradient. This was contrary to my original hypothesis that faster growth and shorter development time would result in smaller adult sizes, as commonly found in ecotherms (Atkinson and Sibly 1997, Nylin and Gotthard 1998, Laurila et al. 2002). Other invertebrate studies have also found little relationship between development time- or predation-stress and adult size (McPeek and Peckarsky 1998, Dmitriew and Rowe 2005), or even report larger adults under time constraints (De Block and Stoks 2004). Invertebrates can have a fixed allometric adult body size by increasing food intake and/or allocation of resources to achieve a particular adult body size, despite variable growth rates (Gurney et al. 2003, De Block and Stoks 2004, Dmitriew and Rowe 2005). Although generalist adult body size may be conserved across disturbance gradients, there may be other associated costs to adult fitness due to rapid juvenile growth such as lower fecundity, reduced dispersal capability (via reductions in flight muscles and storage fats), or limited immune responses (Stevens et al. 2000, Metcalfe and Monaghan 2001, Stoks et al. 2005a). I was unable to measure fecundity due to both species having delayed egg production, but the highly

conserved adult body size across the disturbance gradient I studied suggests there were strong evolutionary benefits to achieving a particular size, such as dispersal capability.

Under unpredictable disturbances there can be a strong incentive for dispersal between habitat types to avoid disturbances and capitalise on habitats during favourable conditions (Hopper 1999, Kisdi 2002). During my study I observed *X. zealandica* adults ovipositing across the entire pond-permanence gradient and consistently found early instar nymphs of both species in short-term temporary ponds. I also have evidence of widespread *X. zealandica* dispersal across the pond-permanence gradient indicated by little genetic differentiation among pond type and lack of any isolation-by-distance relationship (Chapter Four). I suspect similar patterns will apply to *S. arguta* because adults rapidly colonised temporary ponds when they refilled.

Collectively, these empirical results and field observations suggest that these two species may use a bet-hedging strategy to exploit a range of habitats. Bet-hedging is a strategy used by adults to spread risk for their offspring across multiple habitat types, enabling some species to persist across habitat gradients (Hopper 1999, McCauley 2007, Wissinger et al. 2009). Bet-hedging behaviour may be advantageous to exploit unpredictable temporary ponds, even if adults are unable to identify high-risk habitats and their offspring experience high mortality. Similar behaviours have also been observed in aquatic invertebrates and amphibians that readily deposit eggs in unsuitable habitats (McPeek 1989, Rudolf and Rödel 2007). Consequently, many temporary ponds and some permanent ponds (for *S. arguta*) are likely to be population sinks, with potentially high offspring mortality in dry years. This mortality is likely to be balanced by wet years, which likely permit these species to complete development across most temporary ponds and contribute more offspring to the permanent ponds where high risk of predation is a main source of mortality, regardless of climate. This

inter-annual variation resulting in unpredictable habitat availability across a disturbance gradient may result in reciprocal source-sink dynamics, encouraging adult bet-hedging behaviours and flexible nymphal growth (Crean and Marshell 2009, Wissinger et al. 2009). Thus, dispersal and meta-population dynamics could be at the heart of the evolution and sustainability of these life-history strategies when disturbance is unpredictable. However, further unpredictable disturbances could result in more volatile population dynamics, limited distributions, or even species losses as has already been found for both specialists and generalists (Korkeamaki and Suhonen 2002, Suhonen et al. 2014).

Implicationsof different life-history strategies

Different generalist life-histories are typically imperfect strategies to counter, or adapt to, abiotic and biotic selection pressures in favour of wider niche breadth across a range of habitats (McPeek 1996, McCauley 2007, Thibert-Plante and Hendry. 2011). As I have found, the extent of generalist life-history flexibility is dependent on their phenology which can influence their distributions across a disturbance gradient. This means no one generalist species can be classified as a "jack-of-all-trades, master-of-none" (Verberk et al. 2010). Generalist species exposed to long periods of unpredictability during their development likely respond to selection pressures to develop plastic responses to habitat variability (Lytle 2001). On the other hand, more opportunistic generalists, with short development requirements, likely have limited ability to adapt to local environmental conditions.

The constraints of different life-history strategies generalist species use to exploit unpredictable habitats could have consequences for their vulnerability to further environmental unpredictability under global changes. Global changes such as variable climate patterns, presence of invasive species, and human habitat-transformations are driving

a shift toward more homogeneous communities, with higher proportions of generalist species (Marvier et al. 2004, Clavel et al. 2011, Le Viol et al. 2012). However, as I show in this study, generalist species vary in their vulnerability and resistance to unpredictable disturbances and predator pressure depending on their life-history strategy. If habitat disturbances become longer in duration or too frequent, for example, conditions could be too costly for a slow strategy and would favour opportunistic 'fast' strategy (Turner et al. 1998). However, organisms that colonise increasingly unpredictable ecosystems might rely on less disturbed population sources which have alternative selection pressures, limiting inflexible generalists. Therefore, there should be caution when assuming generalists occupying similar habitat gradients will have uniform responses to additional disturbances. Future studies should consider how variations in life-history flexibility and constraints of different generalist strategies may influence their distributions across disturbance gradients. This study provides insight into how alternative life-history strategies enable generalists to achieve broad distributions in heterogeneous freshwater habitats, but also highlights how their resilience and flexibility to local selection pressures is dependent on the constraints of their relative phenologies.

Plate 6. Recently rewetted margins of a semi-permanent pond, part of the Ō Tū Wharekai wetland system, within the Hakatere Conservation Area**.**

Chapter Three:

Flexible and inflexible generalist life-history strategies when exposed to contrasting predator and drying stresses.

Abstract

How generalist species are able exploit heterogeneous habitats likely revolves around how their life-history strategies confer resilience to multiple environmental selection pressures. To counter or adapt to local conditions, generalists likely use a variety of life-history strategies, with both flexible and inflexible developmental and behavioural traits. I investigated the life-history strategies of two aquatic generalist invertebrates, *Xanthocnemis zealandica* damselflies and *Sigara arguta* waterboatmen, which inhabit ponds varying in habitat drying and predator presence (i.e. across a predator-permanence gradient). Through a series of mesocosm experiments with temporary- and permanent-pond nymphs, I determined the flexibility of predator avoidance and drying resistance in each species. *X. zealandica* had flexible behavioural traits, with permanent-pond nymphs better able to evade fish compared to temporary-pond nymphs due to their reduced movement and increased refuge use. Permanent-pond *X. zealandica* also had slower growth than temporary-pond nymphs, but this growth was not influenced by predator presence. Yet, *X. zealandica* also had a fixed response to drying stress with high survival rates (80-90%) during short drying periods (2-8 days), regardless of their natal habitat. In contrast to *X. zealandica*, *S. arguta* had an inflexible life-history with no differences in predator avoidance between permanent and temporary-pond nymphs, and a complete inability to survive drying, regardless of source. However, *S. arguta* had overall higher growth rates than *X. zealandica*. Thus, *S. arguta* may counter potentially

high costs of predation in permanent ponds and drying mortality in temporary ponds through rapid development and terrestrial dispersal between habitats. *X. zealandica*'s flexible life-history is likely driven by their longer nymphal development which requires adaptation to predator and drying stresses to complete their life-cycle. Overall, these two species exemplify how generalists can strongly differ in the life-history strategies they use to counter, or adapt to, local conditions but still have similar persistence across a range of habitats.

Introduction

Organisms that exploit a variety of environments generally adopt life-history strategies which maximise fitness despite variable abiotic and biotic conditions (Van Tienderen 1991, Abrams et al. 1996). However, their ability to adapt to local selection pressures may depend on whether they have fixed or flexible life-history strategies. The fixed strategy can use the same inflexible developmental, behavioural, or morphological phenotypes across multiple habitats regardless of potentially high mortality costs in less suitable habitats (McPeek 1996, Greig and Wissinger 2010). Alternatively, plastic phenotypes can be induced by particular habitat conditions and these flexible life-histories can outperform alternative phenotypes to minimise fitness costs (McPeek 1996, Nylin and Gotthard 1998, Relyea 2002, Hoverman and Relyea 2007). The flexible strategy may be presumed to be superior, but there are also costs associated with plasticity such as: information acquisition (e.g., predator detection), production to build the necessary phenotype (e.g., grow anti-predator spines), maintenance of sustaining sensory and response pathways that induce the plastic responses, and genetic costs (DeWitt et al. 1998, Relyea 2002). These costs could be potentially expensive if there are unreliable environmental cues and the plastic strategy results in the organism producing a maladapted phenotype (DeWitt et al. 1998). The relative costs and benefits of different life-

history strategies will be particularly important in variable freshwater environments where organisms encounter a variety of abiotic and biotic stressors.

Aquatic organisms that occupy a range of freshwater habitats are exposed to a variety of environmental stressors which can influence their ability to adapt to contrasting local conditions. The predictability of abiotic stressors (e.g., habitat drying, catastrophic flooding) can influence the variability and importance of biotic conditions, including: predator assemblages, resource availability, or competition (Wellborn et al. 1996, Williams 1996, Wissinger et al. 2003, Bogan et al. 2013). To explain how hydrologic permanence can result in trade-offs and mortality risks for wetland organisms, Wellborn et al. (1996) proposed a predator-permanence gradient model. Under this model, drying risk in temporary wetlands usually favours species with rapid development to a terrestrial adult stage or species which can resist desiccation (Batzer and Wissinger 1996, Altwegg 2002). In contrast, permanent wetlands, where predation risk is higher (e.g., because fish are present) favour organisms with low activity rates to avoid predator detection at the cost of reduced foraging and subsequent slower development (McPeek 1990, Van Buskirk 1998, Johansson et al. 2001, Altwegg 2002, Relyea 2002, Johansson and Suhling 2004).

The developmental, behavioural, and morphological traits best suited for one habitat type can make them particularly vulnerable in the other, which can result in life-history trade-offs that influence their niche-breadth (McPeek 1990, Stoks and McPeek 2003). Many empirical studies of wetland anuran and invertebrate communities support the predator-permanence model, and it has been used to explain species replacements across wetland permanence gradients (Wissinger et al. 1999, Urban 2004, Lind and Johansson 2007, Richter-Boix et al. 2007). Despite this fitness trade-off, some generalist anurans and macroinvertebrates are able to sustain populations across the predator-permanence gradient, occupying both temporary

and permanent habitats (McPeek 1996, Van Buskirk 2003, Baber et al. 2004, Werner et al. 2007, McCauley 2008, Wissinger et al. 2009). Flexible life-history traits, higher resilience to selection pressures, or an ability to disperse between habitats may all be involved (McCauley 2007, Greig and Wissinger 2010, Franch-Gras et al. 2014), but whether generalists use one or a combination of these strategies to exploit such a wide range of habitats in spite of the fitness trade-offs is poorly understood.

Strategies that allow generalists to balance contrasting fitness trade-offs across the predator-permanence gradient may depend on their life-history flexibility. One strategy likely involves fixed traits such as rapid development, early dispersal between habitats or high fecundity to counter high mortality rates (Batzer and Wissinger 1996, Williams 1996, Verberk et al. 2008a). Alternatively, organisms might rely on flexible life-histories which allow adaptation to local habitat conditions through phenotypically-plastic development rates, variable morphology, flexible behavioural traits or resilient/diapausing life-stages (Batzer and Wissinger 1996, Arnqvist and Johansson 1998, Johansson et al. 2001, Altwegg 2002, Relyea 2002, Richter-Boix et al. 2007 and 2011). These alternative life-history strategies might allow different generalists to occupy a range of habitats, but it is important to understand whether the benefits of flexible and inflexible strategies can offset the potential costs incurred across a range of habitats. To investigate how generalists balance trade-offs, I investigated whether generalist aquatic invertebrates had flexible responses to predator and drying stresses they experienced during juvenile development along a pond-permanence gradient.

New Zealand lentic habitats are an ideal system to test such generalist flexibilities to contrasting environmental selection pressures because these habitats include permanent lakes containing predatory fish and fishless temporary ponds with dynamic hydrologic regimes. Moreover, New Zealand freshwater ecosystems are notable for their high proportion of

generalist species, which has been attributed to weak predation pressure in permanent habitats and highly variable inter- and intra-annual precipitation patterns influencing disturbed or temporary habitats (Winterbourn et al. 1981, Wissinger et al. 2009, Storey and Quinn 2011, Greig et al. 2013).

To test generalist responses to different predators and drying conditions they encountered in permanent and temporary ponds I conducted a series of mecososm experiments to determine the costs and benefits of varying flexibility associated with: 1) predator avoidance, 2) predator-influenced growth, 3) predator-influenced behaviour, and 4) desiccation tolerance. I used two generalist species, a damselfly (Coenagrionidae: *Xanthocnemis zealandica* McLachlan) and a waterboatman (Corixidae: *Sigara arguta* White) which are widespread and common throughout the New Zealand's permanent lakes and temporary ponds (Rowe 1987, Young 2010). I predicted that these generalists have flexible life-history responses to their natal habitat conditions across the predator-permanence gradient. Specifically, I predicted generalists from permanent habitats should show predator-avoidance behaviours (i.e. reduced activity and increased refuge-use) which may result in slower growth and inability to tolerate drying. In contrast, generalists from temporary habitats were expected to benefit from faster growth and desiccation resistance to counter unpredictable drying stresses, but suffer higher mortality when exposed to fish predators due to their higher activity.

Methods

Study species and source habitats

Both generalist species I studied inhabit a wide variety of lakes, temporary ponds, and slow-flowing streams throughout New Zealand from sea level to alpine regions (Rowe 1987, Young 2010). *Xanthocnemis zealandica* are predatory, feeding on zooplankton and small

macroinvertebrates, and can have variable development periods ranging from a year in temporary ponds (Crumpton 1979, Chapter Two) to two-to-three years in permanent lakes (Deacon 1979, Chapter Two) . Nymphs emerge in late austral spring and early summer (November-January), and terrestrial adults can disperse between habitats until late autumn (April-May) (Rowe 1987). *Sigara arguta* are omnivorous, feeding on algae, zooplankton and small macroinvertebrates, and have at least one generation between late spring to autumn (October-May), with adults overwintering in permanent lakes and filled temporary ponds. Adult *S. arguta* have fully-developed wing structures and fly between aquatic habitats, but only feed and mate in aquatic habitats (Young 2010).

The majority of my experiments were located at University of Canterbury's Cass field station (43°2'6.21"S, 171°45'33.28"E, 573 m a.s.l.) situated in the upper Waimakariri River catchment in the South Island high-country. The field station is located within a fluvio-glacial landscape where a variety of permanent and temporary lentic habitats have formed (Gage 1977). The majority of permanent habitats are dominated by small (< 200 mm) native fish (koaro [*Galaxias brevipinnis*] and upland bullies [*Gobiomorphus breviceps*]) and predatory invertebrates (Odonata: *Procordulia grayi* and *P. smithii*) (Wissinger 2006b, Greig 2008). These permanent habitats can also have low densities of larger fish including, native longfin eels (*Anguilla dieffenbachia*), introduced brown (*Salmo trutta*) and rainbow trout (*Onchorynchus mykiss*) (Kelly and McDowall 2004), although trout introductions appear to have had little influence on benthic invertebrate communities in New Zealand high-country lakes (Wissinger et al. 2006). In ponds with inter-annual drying, *P. grayi* and *P. smithii* were the dominant predators (Greig 2008). On the opposite end of the permanence gradient, temporary ponds experiencing aseasonal drying were dominated by small (>10 mm) predatory beetles (Dytiscidae) and water bugs (Notonectidae) (Greig 2008, Wissinger et al. 2009). For all the mesocosm experiments, I sourced *S. arguta* and *X. zealandica* from two

representative habitats, a small permanent lake, Lake Sarah (43°2'56.88"S, 171°46'34.52"E, 577 m a.s.l., 20 ha) and a temporary pond, Bee 1, (43°3'58.37"S, 171°47'24.73"E, 620 m a.s.l., 0.2 ha) which experienced short-term drying (7-14 days), typically in late summer/early autumn. These sites were selected based on their high abundance of the target species throughout the duration of the experiments. Due to inter and intra-annual variability in temporary pond hydrology, consistently sourcing target species across multiple temporary ponds for the duration of many experiments was not practicle. Therefore, source habitats were identified during a multi-year field survey that represented contrasting hydrologic regimes and predator assemblages (Chapter 2). Late instar *P. grayi* dragonfly nymphs were also collected from Lake Sarah to use in the predator experiments. I collected the *S. arguta*, *X. zealandica* and *P. grayi* through repeated D-net (1 mm mesh) sweeps through the littoral zones. Although Lake Sarah contained upland bullies (*Gobiomorphus breviceps*), for the predator experiments I sourced these fish from another nearby lake, Lyndon Tarn (43°19'30.13"S, 171°41'7.94"E), that contained higher densities which were easily collected using Gee minnow traps. All invertebrate and fish identifications were confirmed with reference to appropriate taxonomic keys (McDowall 2001, Winterbourn 2006, Young 2010).

Predator avoidance experiments

To test whether the two generalist invertebrates had flexible predator avoidance behaviours, I measured their mortality rates when exposed to predatory fish and predatory invertebrates. In two-by-three, factorial-design, mesocosm experiments run separately for each species, I collected nymphs from either the permanent or temporary ponds (source treatment) in groups of either 25 *X. zealandica* nymphs (4-6 instar; $\bar{x}$ ± standard deviation [S.D] body size, temporary: 10.28 mm ± 2.16, permanent: 10.33 mm ± 1.94) or 25 *S. arguta* nymphs (4-5

instar; temporary: 4.58 mm ± 1.69, permanent: 4.43mm ± 1.68). These nymphs were placed into a container (40 L, 34 x 54 cm bottom area) randomly assigned one of three predator treatments (upland bullies, dragonflies, or predator-free control), with five replicates of each treatment combination. Each container included 38 L well water, aeration via an airstone, 2-3 cm gravel substrate, two 15-cm tall plastic plants, half a terra cotta pot (10 cm x 9 cm) as refuge for the predators, and a dark shade-cloth lid. The groups of *X. zealandica* were allowed three hours to acclimate to their container before predators that had been starved for 24 hours were added. Predator treatments, either three upland bullies ($\bar{x}$ ± S.D. body size: 64.37 mm ± 6.31) or five late-instar dragonfly nymphs (21.80 mm ± 1.87), reflected densities common in many of the permanent habitats (Staples 1975, Deacon 1979). Following predator additions (*X. zealandica*: 72 hours, *S. argura* 24 hours), predators were removed and each container was searched for surviving generalist nymphs to measure mortality rates. *Sigara arguta* mortality rates were assessed for a shorter time period due to their smaller body size.

Predator-influenced growth experiments

Flexible and inflexible predator-avoidance strategies to different predators might have affected growth; therefore, I ran a mesocosm experiment measuring growth of temporary and permanent pond generalists exposed to predatory fish and invertebrate cues. In separate *X. zealandica* and *S. arguta* experiments, I measured the growth of individually-caged temporary- and permanent-pond nymphs exposed to different predator treatments (upland bullies, dragonflies, controls). The 40 L containers were set up as in the predator avoidance experiments, but also included five individually caged nymphs nested within each predator container. There were five replicates of each habitat/predator treatment combination;

however, the predator densities were reduced (two upland bullies and three dragonflies) to limit the number of prey needed to feed the predators.

Each clear plastic cage (1.26 L, 78 cm^2 bottom area) had two 300-µm mesh windows (4 x 5 cm), a lid with a 5-cm diameter mesh (500 µm) opening, and was provisioned with a 10-cm bamboo stick for habitat structure, and either 20 mL of *Daphnia* prey (approx. 100-150) for *X. zealandica* or 20 mL filamentous algae for *S. arguta*. Cages were suspended on the water surface using 3-cm thick polystyrene rings around the lid. The clear plastic and mesh openings of the cages allowed the invertebrates to experience visual and chemical cues in larger container that held the predators. Over the course of each experiment (*X. zealandica*: 30 days, *S. arguta*: 15 days) the predators were fed con-specific nymphs (25 *X. zealandica* or 50 *S. arguta*) every 48 hours for upland bullies, and 96 hours for dragonflies, to ensure any cues associated with prey consumption were produced by the predators. The *S. arguta* experiment was run for a shorter period due to their faster development rate (Chapter Two) and the predators were fed more nymphs because they have smaller body sizes.

Each individual nymph was photographed to measure body length before being transferred to a cage. Five cages, each with an individual nymph from the same natal habitat were added to each 40 L container and randomly assigned a predator treatment. At the conclusion of the experiment the final body size of each caged nymph was photographed and measured. Growth rates for each species were calculated by converting the initial and final body lengths to dry mass (DM) using length-mass regressions (*X. zealandica*, mg DM = $0.0144L^{2.30}$, Stoffells et al. 2003; and *S. arguta*: mg DM = $0.0098L^{3.05}$, Greig 2008; where L is length in millimetres, I used DM values to estimate instantaneous growth rates (*IGR*) from changes in biomass over a given time interval (T): $IGR = \ln(b_2/b_1)/T$, where b_1 and b_2 are the initial and final biomass, respectively, over the experiment period (Huryn 2002, Galatowitsch and

Batzer 2011). Two temperature loggers (Onset HOBO pendant loggers UA-002-64, Bourne, MA, USA) in separate observation containers were used to continuously measure water temperature throughout the experiment.

Anti-predator behaviour experiments

To determine whether permanent- and temporary-pond nymphs of both generalist species changed their activity and refuge-use when different predators were present I undertook a series of observations [adapted from Johansson (2000) and McCauley (2008)]. The experiment was a two by three repeated measures design with ten replicates, where responses of permanent and temporary pond nymphs were observed in three predator treatments (upland bullies, dragonflies, and non-predator controls, during day and night (repeated measure). Late instar *X. zealandica* ($\bar{x}$ ± S.D. body size, temporary: 12.94 mm ± 0.75, permanent: 13.37 mm ± 0.91) and *S. arguta* (temporary: 4.78 mm ± 0.56, permanent: 4.43 mm ± 0.44) were collected from both source ponds. I selected larger sizes because older nymphs potentially had adapted their anti-predator behaviours to their natal habitat conditions so were more likely to reflect local responses.

All observations were conducted inside a building with ambient temperature ($\bar{x}$ ± S.D. water temperature= 17.8^{o} C ± 3.88) and timer controlled day-length (14 h day/10 h night). Due to the large number of treatment combinations and replicates, trials were staggered over a 10-day period, and treatments were orthogonally assigned to days to ensure that all treatment combinations were represented. Two HOBO pendant temperature loggers were used to continuously measure water temperature in separate observation containers throughout the experiment.

I conducted the experiment in clear plastic containers (7 L, 35 x 23 cm bottom area, 12 cm height) subdivided into two chambers; 3-L section to house predators, and a 4-L arena to observe nymph behaviour. The predator chambers held either one upland bully ($\bar{x}$ ± S.D. body size: 55.60 mm ± 7.13), two dragonfly nymphs (20.27 mm ± 1.90), or a non-predator control. A clear plastic divider separated the chambers with two 5-cm diameter 500-µm nitex mesh openings to ensure any cues associated with predator presence were detected. The container bottom was covered with 1-cm of sand and filled with well water. Each predator chamber, including the controls, had half a terra cotta pot as a refuge, aeration from an airstone, and either 5-late instar *X. zealandica* or 10-late instar *S. arguta* as prey. The observation chamber contained an individual nymph, a centrally-located single 15-cm tall plastic plant as a potential refuge from predators and food (20-30 *Daphnia* for X. *zealandica* or a 2 cm^3 clump of filamentous algae for *S. arguta*). The outside walls and lid of the observation chamber were divided into a 2-cm grid to record the position of the nymph in three-dimensional space. The predators and nymph were added at the same time, and were allowed 24 h to adjust to conditions before observations. Thirty minutes before initial observations *X. zealandica* were fed a further ten *Daphnia* and the fish received an additional three *X. zealandica* or five *S. arguta*. During the day-time observation period nymphal location and refuge use were noted every twelve minutes over two hours for a total of ten observations. The same observations were repeated twelve hours later at night using a red LED light. Red light was used to avoid startling the nymph, as in other aquatic invertebrate behaviour studies (Hampton 2004). Following each observation trial, all animals were removed, containers were washed, dried, and the sand was replaced. Each container retained its assigned predator treatment to avoid mixing different predator chemical cues between containers.

For each observational period a nymph's Euclidean distance (*d*) moved through three-dimensional space (*x*-,*y*-, and *z*-axes) was calculated between successive 12-minute observations using: $d = \sqrt{(|\mathrm{x}[1] - \mathrm{x}[2]|^2 + |\mathrm{y}[1] - \mathrm{y}[2]|^2 + |\mathrm{z}[1] - \mathrm{z}[2]|^2)}$. The sum of these distances was used as the total distance moved over the observation period.

Desiccation stress experiments

To determine if generalist invertebrates developing in temporary ponds were more resilient to drying stress, I compared survival of nymphs from both source habitats over various lengths of drying using microcosms [adapted from Wissinger et al. (2003), Galatowitsch and Batzer (2011)]. In February 2013 I collected early instar *X. zealandica* ($\bar{x}$ ± S.D. body size, temporary: 6.71 mm ± 0.69, permanent: 6.11 mm ± 0.75) and late instar *S. arguta* (temporary: 5.71 mm ± 0.29, permanent: 5.52 mm ± 0.36) from Lake Sarah and Bee 1 pond. These instars are the most common life-stages for nymphs in temporary ponds during the austral summer and early autumn (February-April) when many of these habitats begin to dry (Chapter Two). The two invertebrates were transported to the University of Canterbury in Christchurch, New Zealand and maintained in a temperature-controlled room (14 h day/10 night, 20^{o} C air temperature). Individuals of both species from each habitat were placed in separate containers (1.26 L; 78 cm^2 bottom area) that included: two centimetres of temporary pond sediment that had been previously collected, dried, and homogenised, 1 L well water, an air-stone for dissolved oxygen, a 10-cm bamboo stick for habitat structure, and 20-mL food containing ostracods for *X. zealandica* (approx. 30-50) and filamentous algae for *S. arguta*. Each container also had a 500 µm-mesh lid to prevent nymphs escaping. Individuals were randomly assigned to six drying treatments (0, 2, 4, 6, 16, 32 days without water) with ten replicates. Original water depths were maintained in the controls, whereas over 48 hours

water was gradually lowered to 1 cm below the sediment in drying treatments. The survival of control individuals was assessed after the initial two-day period.

Drying treatments remained without water for the prescribed period, and prior to rehydration, a three-centimetre core of sediment was removed to measure sediment moisture. Sediment cores were weighed, retained in separate weighing tins, and rehydrated with well water. The experimental containers were filled with 1 L water and 24 hours later each experimental container and its corresponding sediment core was examined to assess whether the nymph survived. The sediment cores were then dried for at least 72 hours at 50^{o}C, cooled in a desiccator for 48 hours, and reweighed to determine percent sediment moisture in each container.

Statistical analyses

I assessed mortality rates of the two generalist species using two separate two-way analyses of variances (ANOVA) comparing survival across source populations (either temporary or permanent) and predator treatments (fish, dragonfly, control) with arcsin-square-root-transformed proportions of nymphs consumed as response variables. Any significant treatment interactions where further analyzed using Tukey posthoc tests.

The non-consumptive predator influences on growth were compared using linear mixed effects models (LME), where source habitat (temporary vs. permanent), predator treatments (fish, dragonfly, control), and their interaction were fixed effects. I included the treatment container as a random effect to control for variations in individual nymphal growth in each container. Separate models for each species were evaluated using lmer from the lme4 (Bates et al. 2013) R package. The lmerTest package was used to summarise the fixed effects using

an ANOVA with Kenward-Roger approximations for degrees of freedom and to calculate p-values, along with log-likelihood ratio tests to assess the random effect (Kuznetsova et al. 2013).

I also evaluated the influence of different predators on temporary- and permanent-pond nymphal behaviour using LME. For each species, separate models were tested for: 1) total Euclidean distance an individual nymph moved during an observation period, and 2) the proportion of time (arcsin-square-root-transformed) the nymph spent in refuge. For each model, the main effects and interactions of habitat type (temporary vs. permanent), predator (fish, dragonfly, control), and observation time (day vs. night) were set as fixed effects. Individual nymphs and trials were included as random effects to account for variation in individuals' behaviour during the two observation periods, and to incorporate the influence of trials performed on different days. By including individual as a random effect I also accounted for the non-independence of the multiple behavioural observations of each nymph through time and ensured effects were tested with appropriate error terms. As in the growth experiment, these models were tested using lmer from the lme4 and lmerTest packages.

To assess desiccation tolerance I compared the survival of nymphs sourced from temporary and permanent habitats using survival analysis with sediment moisture as a co-variant. The Cox model, within the package SURVIVAL (Therneau 2013), allowed me to contrast habitat differences and assess how reduced sediment moisture over longer drying treatments influenced nymph survival (Therneau and Grambsch 2000). Because *S. arguta* had no surviving nymphs in the drying treatments this analysis was only applied to *X. zealandica*.

I used R version 3.0.2 (R Development Core Team 2013) for all experiment analyses and for each test I found no deviations from homoscedasticity or normality for the residuals of transformed response variables.

Results

Predator avoidance experiments

Both generalist species were more susceptible to visually-feeding predatory fish (*X. zealandica*: $F_{2,24} = 207.79$, $p < 0.001$; *S. arguta*: $F_{2,24} = 162.07$, $p < 0.001$) than sit-and-wait dragonflies (Figure 3.1). However, for *X. zealandica* there was a significant interaction between predator type and source habitat ($F_{2,24} = 4.95$, $p < 0.01$; Figure 3.1A), primarily due to slightly higher mortality of temporary-pond nymphs exposed to bullies than those from the permanent pond. In contrast, *S. arguta* did not have significant interactions between predator type or source habitat ($F_{2,24} = 0.58$, $p = 0.57$) and there was no effect of source pond ($F_{1,24} = 3.53$, $p = 0.07$; Figure 3.1B).

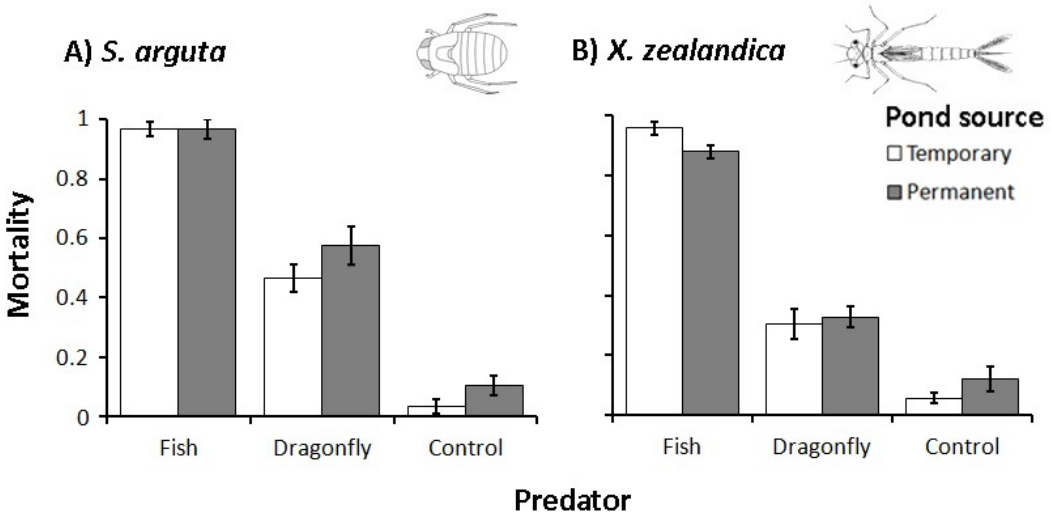

Figure 3.1. Mortality (proportion, mean ± S.E.) due to consumptive affects of two predators, fish (*Gobiomorphus breviceps*) and dragonflies (*Procordulia grayi*), and a predator-free control on *X. zealandica* (A) and *S. arguta* (B) nymphs from temporary and permanent ponds.

Predator-influenced growth experiments

There was no significant difference in growth between predator treatments for either generalist species (*X. zealandica*: $F_{2,21.8} = 0.17$, $p = 0.84$; *S. arguta*: $F_{2,23.7} = 0.59$, $p = 0.5$; Figure 3.2). However, non-consumptive effects on growth were 55.5% higher for temporary-pond *X. zealandica* than permanent-pond nymphs ($F_{1,21.8} = 6.88$, $p = 0.016$; Figure 3.2B). Despite the significant interaction effect in the predator avoidance experiment previously described (Figure 3.1), there was no predator by habitat interaction influence on *X. zealandica* growth ($F_{2,21.8} = 2.23$, $p = 0.13$; Figure 3.2B). *Sigara arguta* growth also did not differ between source habitat ($F_{1,23.7} = 0.15$, $p = 0.70$), and there was also no interaction between predator type and source habitat ($F_{2,23.7} = 2.89$, $p = 0.07$; Figure 3.2A). *S. arguta* also had higher mean (± S.E.) growth rates (0.052 mg/d ± 0.001) than *X. zealandica* (0.013 mg/d ± 0.001) irrespective of source habitat or predator presence (Figure 3.2).

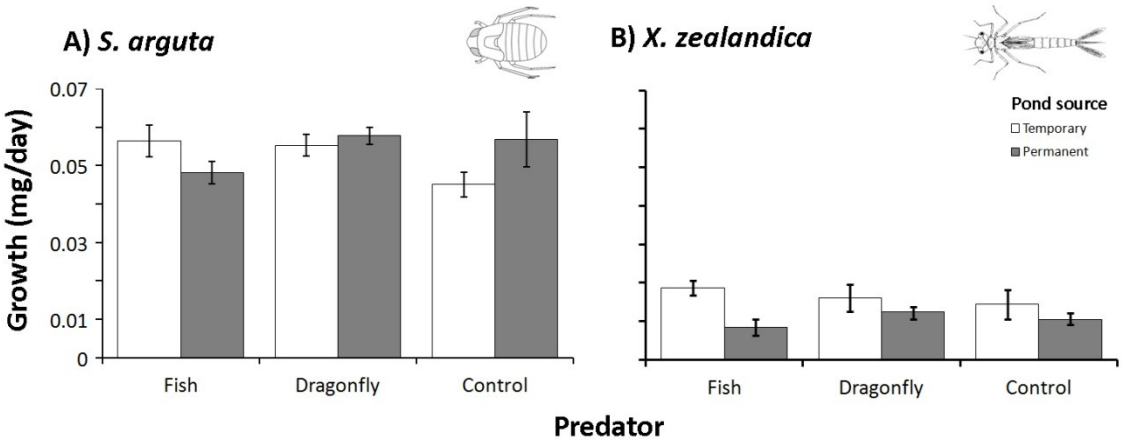

Figure 3.2. Body mass growth (mean ± S.E.) of *X. zealandica* (A) and *S. arguta* (B) nymphs from temporary and permanent ponds due to non-consumptive effects of predators (fish [*Gobiomorphus breviceps*] and dragonflies [*Procordulia grayi*]) and a predator-free control.

Anti-predator behaviour experiments

Behavioural responses to predators varied between the two generalist species and depended on source habitat (Figure 3.3). Temporary-pond *X. zealandica* moved greater distances overall than permanent-pond nymphs ($F_{1,51}$ = 5.33, p = 0.03). *X. zealandica* nymphs also reduced their total distance moved when exposed to different predators, particularly in the fish treatments ($F_{2,51.3}$ = 3.36, p = 0.04). *X. zealandica* nymphs also had overall greater movements at night than during the day ($F_{1,54}$ = 21.86, $p < 0.001$; Figure 3.3C). In addition to reduced movement, permanent-pond *X. zealandica* spent substantially more time in refuges ($F_{1,51.2}$ = 7.55, p = 0.008) regardless of predator type ($F_{2,52.6}$ = 0.38, p = 0.68) or time of day ($F_{1,54}$ = 1.68, p = 0.20; Figure 3.3D).

In contrast, *S. arguta* movements did not differ between habitat source ($F_{1,52}$ = 0.05, p = 0.82) or predator type ($F_{1,53.3}$ = 0.52, p = 0.59), but they were more likely to move at night than during the day ($F_{1,54}$ = 4.98, p = 0.03; Figure 3.3A). Similar to *X. zealandica*, their time spent in refuges was significantly higher at night ($F_{1,54}$ = 6.38, p = 0.014) regardless of predator type ($F_{2,52.4}$ = 0.06, p = 0.94) or source habitat ($F_{1,52}$ = 0.24, p = 0.63; Figure 3B). There were no significant interactions for either total distance moved or proportion of time spent in refuges for either species.

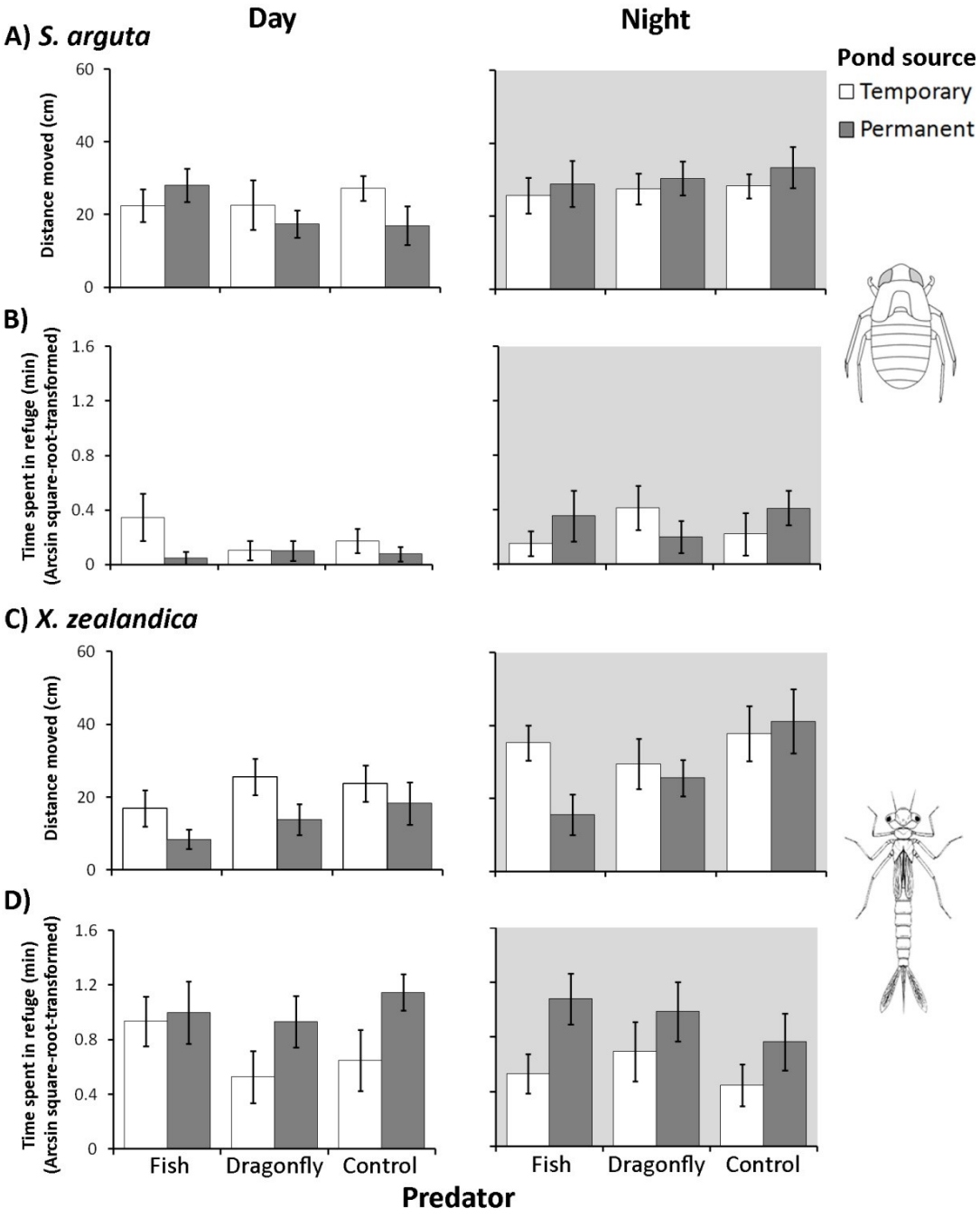

Figure 3.3. Total Euclidian distance moved (A and C; mean ± S.E.) and total time spent in refuges (B and D; mean ± S.E.) during 2-hour day and night observation periods when temporary- and permanent-pond *X. zealandica* and *S. arguta* were exposed to caged predatory fish (*Gobiomorphus breviceps*), dragonflies (*Procordulia grayi*), or a no-predator control.

Desiccation stress experiments

Xanthocnemis zealandica was resilient to drying stress, while *S. arguta* was much more susceptible to desiccation. *Xanthocnemis zealandica* had 80-90 % survival up to eight days without water and 10-20 % survival after 16 days without water (Figure 3.4). However, there was no significant differences between source habitat type affecting *X. zealandica* (Cox model: z = -0.32, p = 0.75). Nevertheless, percent substrate moisture (z = 2.47, p = 0.013) was a better predictor of *X. zealandica* survival. In contrast, *S. arguta* were unable to survive any period of drying.

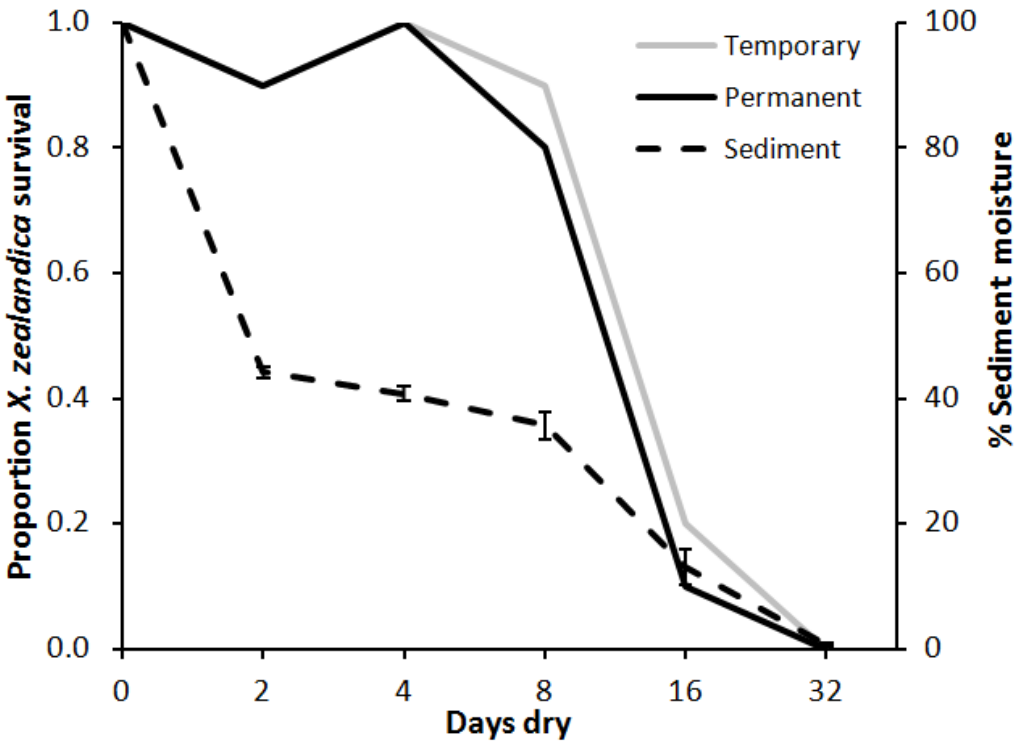

Figure 3.4. Proportion of temporary- and permanent-pond *X. zealandica* surviving in six drying treatments (solid dark and gray lines), and the percent sediment moisture (mean ± S.E.), remaining in the mesocosms before rehydration (dotted line).

Discussion

Unpredictable ecosystems can favour generalist species that have life-history strategies to withstand a variety of abiotic and biotic selection pressures (Van Tienderen 1991). However, not all generalist species use flexible life-history strategies to exploit a range of habitat types (McCauley 2008, Verberk et al. 2008a, Greig and Wissinger 2010, Storey and Quinn 2011). How these species are able to exploit multiple habitat types may depend on their ability to balance the costs and benefits of adapting to local conditions given their non-specialised strategy. I investigated whether flexibility to local environmental stresses was the key to how generalists sustained broad realised niches. My hypothesis that generalists would have flexible behavioural and developmental responses depending on their natal habitat conditions was partially supported through *X. zealandica*'s habitat-dependent reaction to predators. However, contrary to my predictions, *S. arguta* had inflexible predator avoidance behaviours regardless of their source habitat. Furthermore, both species had fixed responses to drying; *X. zealandica* nymphs from the two habitat types had similar desiccation tolerance, while *S. arguta* had no tolerance to drying whatsoever. These contrasting flexible and inflexible life-histories of *X. zealandica* and *S. arguta,* respectively, reveal these two generalist species had quite different strategies to exploit the same variety of habitats occurring across a pond predator-permanence gradient. Understanding the fitness benefits and costs of these different generalist life-history strategies will underpin how species respond to variable environmental conditions and will likely reveal the limitations of these strategies.

Alternative strategies of generalists for predator avoidance

Flexible life-history: Xanthocnemis zealandica

The more cautious behaviours (e.g., reduced movement and greater refuge use) of permanent-pond *X. zealandica* may have reduced encounters with fish in the predator consumption experiment and account for their reduced mortality rates. This difference between nymphs sourced from permanent and temporary ponds suggests *X. zealandica* have flexible anti-predator responses. Behavioural studies of invertebrates that coexist with fish indicate prey usually either reduce activity, spend more time in refuges, or reduce their foraging effort, resulting in reduced growth rates (Dixon and Baker 1988, McPeek 1990, Peckarsky 1996, Johansson 2000, Stoks and De Block 2000, Johansson and Suhling 2004, McCauley 2007). Based on the anti-predator behaviours and lower mortality rates of permanent-pond *X. zealandica*, I expected a corresponding reduction in growth-rate in response to fish presence. However, I observed no effect of predators on *X. zealandica* growth, which was consistent with growth rate patterns observed in the field (Chapter Two). My results suggest that *X. zealandica* anti-predator behaviours and development rates may be established earlier in their development and could be fixed for the duration of their nymphal stage. The fixing of these phenotypes in early development may be a phenotypic response to predator cues or a selection of individuals through predator consumption of maladapted nymphs, which could be tested through egg or earlier-instar responses to environmental cues (McIntosh and Townsend 1994, Johansson et al. 2001, Stoks et al. 2005b).

In contrast to the anti-predator behaviours in permanent habitats, many temporary-pond invertebrates and amphibians have incautious behaviours associated with higher foraging rates and accelerated development which allows them to achieve a terrestrial adult stage before the habitat dries (Anholt et al. 2000, Johansson and Suhling 2004, Richter-Boix et al.

2007, Verberk et al. 2008b, Greig and Wissinger 2010). Along these lines, temporary-pond *X. zealandica* used refuges less and moved more despite the presence of predators. As in other studies, these behaviours can make invertebrates and amphibians more susceptible to predatory fish that rely on visual cues to detect prey (McPeek 1990, Relyea and Werner 1999, Stoks and DeBlock 2000). Therefore, these incautious behaviours likely explain why temporary-pond nymphs were consumed more by fish than their permanent-pond counterparts. However, their behaviours may not be at a particular disadvantage against ambush invertebrate predators (e.g., dragonflies, beetles) dominating temporary ponds because they could more easily swim away as *Enallagma* damselflies do (McPeek 1990, Stoks and DeBlock 2000). Overall, these results indicate that *X. zealandica* mediate predation risks through flexible behaviours and development across the predator-permanence gradient.

Inflexible life-history: Sigara arguta

In contrast to *X. zealandica*'s strategy, I uncovered no evidence of a flexible life-history strategy in *S. arguta*. There were no habitat-dependent differences in predator avoidance, growth rates, or anti-predator behaviours. *Sigara arguta* were also the most susceptible to consumption by fish. Their high rate of movements and low refuge-use, irrespective of predator presence, likely made them easily noticeable to visually-feeding predatory fish explaining their higher mortality rate compared to *X. zealandica*. Similar risky predation behaviours have been observed in other aquatic invertebrates (*Baetis* spp. mayflies and *Enallagma* spp. damselflies) that require increased foraging to maintain high metabolism and growth for short juvenile development (McPeek 1990, Peckarsky 1996, Stoks and De Block 2000). Nevertheless, the short development periods attained by *S. arguta*, meaning they achieve adult dispersal early, may balance risky juvenile behaviours thereby reducing their

overall exposure to predators. Although *S. arguta* did not use refuges in the mesocosms, they frequently use shallow lake margins which likely provide a sub-habitat refuge from predatory fish (Chapter Two). Furthermore, permanent ponds without these shallow margins have few *S. arguta* nymphs or adults (Chapter Two). In concordance with these explanations, a study following fish introductions in ponds found corixids experienced high mortality and their distributions were reduced exclusively to shallow littoral margins (Oscarson 1987). Although *S. arguta* have behaviours making them vulnerable to fast-swimming fish, they were better at eluding dragonflies which are ambush predators. In semi-permanent habitats where dragonflies are the top predators, *S. arguta* were widespread and abundant (Chapter Two). Thus, overall *S. arguta* may experience high mortality from predatory fish due to inflexible behaviours, but they likely trade-off predation risk with rapid development and limit their predator exposure through use of sub-habitat refuges. These inflexible responses likely mediate the risks associated with occupying permanent habitats, resulting in weak selection pressures that do not promote more specialised anti-predator adaptations, ultimately facilitating the very wide realised niche they occupy.

Alternative fitness strategies of generalists for desiccation risks

Habitat drying can be a particularly harsh abiotic stress for aquatic organisms (Batzer and Wissinger 1996, Bogan and Lytle 2011, Richter-Boix et al. 2011). Strategies to tolerate or avoid drying (e.g., desiccation tolerance, rapid growth and terrestrial dispersal) can be a challenge under unpredictable hydrology because aquatic organisms maybe caught at vulnerable life-stages and experience high mortality (Wissinger et al. 2006, Rudolf and Rodel 2007, Verberk et al. 2008b, Storey and Quinn 2011, Bogan et al. 2013). Despite the importance of drying influences on freshwater populations and communities, few studies

have actually empirically tested the desiccation tolerance of aquatic macroinvertebrates (Wissinger et al. 2003, Kosnicki 2005, Rebora et al. 2007, Galatowitsch and Batzer 2011, Wickson et al. 2012). I had predicted temporary-pond generalist nymphs would have adaptations to withstand pond drying, whereas permanent-pond nymphs would have less resilience to drying stress. However, as with the response to predation, the two generalist species had starkly different reactions to drying. *Xanthocnemis zealandica* had short-term desiccation tolerance regardless of their natal habitat types, whereas *S. arguta* were completely intolerant of drying.

Eighty-five percent of *X. zealandica* survived eight days of drying and fifteen percent were able to survive 16 days dry at lower sediment moistures. Moreover, it is conceivable that *X. zealandica* may be able to withstand even longer dry periods in the field if sediment moisture content is high. Similar to other studies of *X. zealandica* (Crumpton 1979, Rowe 1987), fourth and fifth instar nymphs were present in temporary ponds that had remained dry for at least a month (Chapter Two). High survival of drying conditions is notable for a non-specialist species, which suggests *X. zealandica* might have evolved physiological responses to resist desiccation that may be linked to their development period.

Xanthocnemis zealandica's tolerance of short-term drying conditions may be a "hardwired" physiological response to avoid desiccation due to long juvenile development periods and also linked with population mixing across temporary and permanent ponds. Because *X. zealandica* has a development period of at least a year, they have a greater likelihood of experiencing temporary-pond drying (Chapter Two). Organisms which experience environmental stress during their development are more likely to evolve resistance traits (Lytle 2001). Additionally, genetic exchange across a range of habitats can retain beneficial traits (Bourne et al. 2014). I have observed adult *X. zealandica* ovipositing in multiple

neighbouring permanent and temporary ponds, and from genetic analyses there is little evidence of genetic differentiation between habitat types (Chapter Four). Therefore, *X. zealandica* may have adapted desiccation tolerance and retained this response even if they never experience drying in permanent habitats. This suggests *X. zealandica* may incur little cost to maintain a desiccation- resistant trait which could otherwise limit their ability to occupy temporary ponds. Due to the limits of their desiccation tolerance, *X. zealandica* can suffer extirpation from temporary ponds with long drying periods (Chapter Two). However, recolonisation from permanent pond sources and successful juvenile development during wet years could balance inter-annual trade-offs of occupying temporary ponds.

Sigara arguta was intolerant to drying, but their rapid juvenile development may limit their exposure to drying stress. Even though pond hydrology varies across the predator-permanence gradient, *S. arguta* experience similar developmental time constraints between habitats. Comparable fixed developmental traits have also been found in organisms that rapidly colonise and complete development before they experience competition or disturbances (Caceres 1997, Chesson and Huntly 1997, Greig and Wissinger 2010). By exploiting short windows of advantageous developmental conditions, organisms with fixed traits can exploit a range of habitats and rely on dormancy or ontogenetic habitat shifts to persist during unfavourable conditions (Caceres 1997, Greig and Wissinger 2010). Therefore, organisms with inflexible strategies like *S. arguta* use rapid juvenile development to counter any potential trade-offs associated with desiccation tolerance versus predator avoidance, meaning unpredictable disturbance and biotic interactions result in weak selective pressures on them to evolve more flexible adaptations.

Consequences of life-history strategies under variable environmental stressors

The various mesocosm experiments had the advantages of rigorous control of predator and drying stresses to detect how these specific environmental conditions influence mortality, growth, and behaviours of generalists with different life-history strategies from contrasting natal habitats. However, due to logistical constraints these experiments were limited to sourcing both study species from a representative temporary and permanent pond, so lacked spatial replication of the source ponds. This is a challenge of many behavioural and developmental studies sourcing organisms from representative environments (McIntosh and Townsend 1994, Johansson et al. 2000, De Block et al. 2008). Nevertheless, the source populations chosen were representative of the particular hydrological conditions. Thus, my results indicate source population did influence the traits tested, and the most likely cause of the differences was probably the hydrological conditions of those ponds.

Overall the patterns from my experiments suggest generalist species can use alternative life-history strategies to balance contrasting selection pressures across an unpredictable disturbance gradient, but flexible responses to stressors may depend on their phenology. My mesocosm experiments revealed that *X. zealandica* and *S. arguta* have very different life-history strategies, one flexible and the other inflexible, despite exploiting the same range of habitats across the pond predator-permanence gradient. Flexible life-history strategies are predicted for organisms coping with environmental variability (Schlichting and Pigliucci 1998). However, the extent of flexibility for organisms with broad realised niches may depend on whether they are likely to encounter environmental variability during juvenile development.

The inflexible and flexible life-history strategies of the two species I studied were consistent with Sih's (1987) division of life-histories into "fast" and "slow" strategies, where "fast"

species are more active and develop faster, making them liable to take more risks but also allowing them to exploit more temporary habitats. However, these same "fast" species can be excluded from permanent habitats as Wellborn et al. (1996) proposed in the pond predator-permanence gradient model. Nevertheless, under unpredictable disturbance regimes the effect of these selection pressures is likely to be quite different. Firstly, my results indicate the "fast" strategy with rapid growth and earlier maturity will experience little selection pressure to develop flexible traits even if the disturbances are frequent and cause high mortality. Secondly, slower-growing organisms will more readily evolve adaptations to cope with unpredictable disturbances (Lytle 2001). Therefore, *X. zealandica*'s flexible life-history strategy was linked to their longer nymphal development, whereas *S. arguta* were less developmentally constrained and would not necessarily benefit from a flexible response to environmental stressors. Thus, both flexible and inflexible generalist life-history strategies might have evolved to reduce the risk of occupying unpredictable environments with contrasting selection pressures.

In multiple models of species evolution in heterogeneous environments, weak selection is critical for the retention of generalist traits across a range of habitats (de Meeus and Goudet 2000, Debarre and Gandon 2011, Massol 2012). Moreover, a combination of weak divergent selection and dispersal can promote the persistence of generalist populations with broad distributions (Nagelkerke and Menken 2013, Bourne et al. 2014). Previous studies of New Zealand pond invertebrate communities have attributed generalist prevalence to low predation pressure in permanent ponds and widespread dispersal between habitats (Wissinger et al. 2009, Greig et al. 2013). Along these lines, although generalist species with flexible and inflexible life-history strategies may experience high mortality from unpredictable disturbances, widespread dispersal across habitat types could also limit the selection pressures to evolve more specialised traits for natal environments. Thus, overall my study

highlights how flexible and inflexible generalist life-history strategies can mediate contrasting selection pressures through different developmental, behavioural, and tolerance traits, enabling these species to persist across a wide range of freshwater habitats.

Plate 7. Pairs of *Xanthocnemis zealandica* adults ovipositing in a dry temporary pond within the Tarndale pond complex. Gene flow via adult dispersal among different pond types might hedge their offsprings' success and sustain populations across the pond-permanence gradient.

Chapter Four:

Dispersal sustains generalist metapopulations across a pond-permanence gradient: population genetic patterns of a damselfly (Odonata: Coenagrionidae: *Xanthocnemis zealandica*).

Abstract

Widespread dispersal may be critical for generalist species to persist across a wide range of habitats by balancing contrasting local selection pressures resulting in metapopulations. The extent of gene flow across heterogeneous environments can influence the genetic diversity, population structure, and resilience of generalists to unpredictable disturbances. I investigated whether *Xanthocnemis zealandica* damselfly genetic variation was influenced by contrasting abiotic and biotic selection pressures across a gradient of ponds with different hydrologic regimes. Using eight microsatellite loci, I tested the effects of ecological factors and potential dispersal barriers on the genetic diversity, divergence, and gene flow among 22 ponds within four pond complexes spread across New Zealand's high-country. I found distinct North and South Island populations, but at lower spatial scales genetic differentiation was more heterogeneous and multifaceted. Genetic diversity was not influenced by pond-permanence and potential ecological drivers of metapopulation dynamics (e.g., pond area, hydroperiod, and presence of predatory fish) were poor predictors of genetic structure. Asymmetrical gene flow patterns within the pond complexes suggest that permanent habitats may act as important sources of dispersers to neighbouring ponds. In addition, there was evidence of long-distance dispersal among pond complexes within the South Island. Overall, my results

show that extensive dispersal among and within metapopulations allow generalists to persist across heterogeneous environments and this gene flow may override ecological influences that typically lead to divergence and evolution in local populations.

Introduction

Environmental heterogeneity can greatly influence dispersal between variable habitats and the subsequent success of local populations. Environmental stability impacts the extent of dispersal between possible habitats, the ability of organisms to adapt to local conditions, and population resilience to disturbance (Holt and McPeek 1996, Holt et al. 2004, Hanski et al. 2011, Shama et al. 2011). These responses in turn affect the local and regional genetic diversity through the exchange of advantageous alleles or genetic divergence between habitats (Manel et al. 2003, Schoville et al. 2012). Under stable or predictable habitat conditions, genetic diversity is likely to increase among habitat types due to adaptation to local environmental conditions (Lytle and Poff 2004). With greater adaptation to local conditions, there may be less incentive to disperse between multiple habitat types and could lead to greater genetic divergence (Lytle 2001, Stoks and McPeek 2006). Alternatively, divergence could also result from dispersal into habitats where these organisms are unable to establish due to environmental mismatches. In unpredictable environments, individuals with poor dispersal capabilities or an inability to adapt to unfavourable habitat conditions may risk local extirpation (Hoehn and Henle 2007). However, if there is dispersal across multiple habitat types, repeated local extinction and recolonisation events could reinforce gene flow and prevent local population differentiation (Slaktin 1977). Therefore, under unpredictable environmental regimes there may be a greater benefit for dispersal across multiple habitat

types balancing extinction and recolonisation events, resulting in metapopulation dynamics (Harrison and Hastings 1996, Hanski 1998, Huey et al. 2011).

Although local extinctions can reduce the overall population size, metapopulation dynamics with immigration from multiple source populations (migrant-pool model) can increase the genetic recovery and may maintain high genetic diversity despite size fluctuations (Berthier et al. 2006, Huey et al. 2011). Limited adaptation and genetic differentiation under unpredictable environmental conditions might reduce speciation and favour generalist species that have wide niches and readily immigrate between habitats (Van Tienderen 1991, Holt and Gomulkiewicz 1997, Huey et al. 2011). There is empirical evidence to show freshwater communities that experience unpredictable disturbances (e.g., temporary streams and ponds) have high proportions of generalist species and likely persist in these habitats via their flexible development, environmental tolerances, or strong dispersal abilities (Winterbourn et al. 1981, Wissinger et al. 2009, Greig 2008, Verberk et al. 2008a).

Aquatic habitats are excellent systems for investigating the role of meta-populations in maintaining regional populations across heterogeneous environmental conditions (Bohonak and Jenkins 2003, Huey et al. 2011). In particular, pond environments can have dynamic habitat drying and flooding events, along with variable predator regimes which influence population success and the connectivity between habitats (Williams 1996). These environmental conditions result in a pond-permanence gradient with annually-drying temporary ponds dominated by invertebrate predators, and permanent ponds that can sustain vertebrate predators (i.e. fish and salamanders; Wellborn et al. 1996, Wissinger 2003). Because juvenile amphibians and invertebrates are usually constrained to the pond boundary they experience local habitat conditions until they are able to terrestrially disperse as adults (Bohonak and Jenkins 2003, Cosentino et al. 2012). Therefore, how juveniles adapt to, or

counter local selection pressures can influence their distributions across pond-permanence gradients and might result in specialised traits for desiccation tolerance or predator avoidance (Wellborn et al. 1996, Wissinger 2003). However, some generalist anurans and invertebrates are able to exploit habitats across the pond-permanence gradient through their high tolerance, phenotypic plasticity, and/or an ability to disperse among habitats (McCauley 2008, Werner et al. 2007, Wissinger et al. 2009).

Dispersal may be especially important to sustain metapopulations of generalists across these pond-permanence gradients (McCauley 2007 and 2008, Verberk et al. 2008a). During particularly long dry periods temporary ponds can be population sinks with subsequent recolonisation from more permanent habitats once the temporary ponds refill with water (De Block et al. 2005, Wissinger et al. 2009). During favourable periods, with low predation risk and resource competition, temporary-pond larvae may be able to complete development and contribute more individuals to regional gene pools than permanent habitats (McPeek 1989). Metapopulation dynamics may be important to sustain generalist populations across unpredictable pond-permanence gradients, but the extent of gene flow between these habitats is poorly understood.

To test how meta-population dynamics influence the spatial genetic structure of a pond generalist species, I used a New Zealand damselfly species *Xanthocnemis zealandica* McLachlan of the family Coenagrionidae. This species is widespread throughout the North and South Islands of New Zealand (Rowe 1987, Nolan et al. 2007) and their obligate aquatic larvae inhabit a range of habitats across the pond-permanence gradient (Crumpton 1976, Wissinger et al. 2009). This habitat gradient included temporary ponds that experience annual drying, semi-permanent ponds that dry inter-annually, and permanent lakes where they coexist with predatory fish [native: upland bullies (*Gobiomorphus breviceps*), koaro

(*Galaxias brevipinnis)* and invasive: rainbow (*Onchorynchus mykiss*) and brown trout (*Salmo trutta*) (Kelly and McDowall 2004)] and invertebrates (*Procordulia grayi* and *P. smithii* dragonflies) (Greig 2008, Wissinger et al. 2009).

Previous life-history studies using field surveys (Chapter Two) and mesocosm experiments (Chapter Three) revealed *X. zealandica* larvae have plastic responses to local environmental conditions. In temporary ponds *X. zealandica* are able to tolerate short dry periods (< 90 days) and are able to complete development in less than a year (Crumpton 1979, Chapter Two). In contrast, permanent pond larvae are better able to avoid fish predators and have slower growth rates with development over two-three years (Deacon 1979, Chapter Two). Terrestrial adults emerge from the ponds in November-February (austral spring and summer) and have been observed dispersing between habitat types along with laying eggs in both filled and recently dried ponds before perishing in late autumn (May-June). The females lay eggs in temporary and semi-permanent ponds, but their developing offspring can experience local extinctions due to unpredictable precipitation that can cause long periods of intra- and inter-annual drying (Greig 2008, Wissinger et al. 2009). Evidence of adult dispersal across the pond-permanence gradient along with plastic adaptations to local habitat conditions suggests that *X. zealandica* could maintain population structure through genetic metapopulations.

To investigate whether dispersal between pond types sustained metapopulations across the pond-permanence gradient, I determined the genetic structure of populations of *X. zealandica* in pond complexes in New Zealand's high-country. High-country pond complexes were clusters of neighbouring pond and lake habitats in mountainous terrain that were formed out of glacial moraines during the Pleistocene (Gage 1977, Wallis and Trewick 2009). Using a range of habitats across the permanence gradient from four pond complexes across New Zealand I examined the scale at which metapopulation dynamics occurred in *X. zealandica*. I

also tested for variable genetic diversity and differentiation due to differences in pond characteristics (hydrology, area, or fish presence). Although *X. zealandica* is potentially a strong disperser (Nolan et al. 2007), I predicted mountain and ocean barriers between pond complexes would limit *X. zealandica* dispersal resulting in individual pond complexes acting as unique metapopulations. Within pond complexes, I hypothesised there would be little differentiation by pond habitat type, but lower genetic diversity due to population turnover in temporary ponds from recurring unpredictable drying. I also predicted greater gene flow from permanent-pond populations to temporary-pond populations resulting from recruitment following population turnover.

Methods

Site locations and specimen collection

To test for genetic differentiation between and among New Zealand high-country pond complexes in the North and South Islands, *X. zealandica* damselfly larvae were collected from different habitats that represented a range of hydrological pond permanence and fish presence (Figure 4.1, Table 4.1). Larvae were used because they were unable to disperse between ponds until they emerge as adults, therefore, the natal habitat was known and the larvae had experienced the local hydroperiod and predator conditions. Because adult damselflies are known to be moderate dispersers (0.1 – 4.5km from natal habitats) between aquatic habitats (De Block et al. 2005, Keller and Holderegger 2013), I chose pond complexes separated by at least 80 kilometers: one complex on New Zealand's North Island (Makirikiri) and three in the South Island (Tarndale, Cass, and Hakatere). The two main islands of New Zealand are separated by the Cook Strait, which is only 22 km wide at its narrowest point. Within each pond complex two permanent, semi-permanent, and temporary

ponds were selected based on continual water depth measurements (North Island: 2009-2011; South Island: 2006-2008, 2010-2013) using Onset water depth loggers (Onset HOBO pendant loggers UA-002-64, Bourne, MA USA); with the exception that no semi-permanent ponds were sampled on the North Island. Permanent ponds were habitats that had no history of drying, semi-permanent only dried in years with particularly low precipitation, while temporary ponds consistently dried annually. *X. zealandica* larvae were collected during the austral summer from the North Island pond complex in January 2011 and the South Island pond complexes in December 2011-February 2012. In each pond, I used successive 1-m sweeps of a D-net (1 mm mesh) through 5-20 littoral and benthic micro-habitats to collect at least 100 larvae. The larvae were separated from the pond detritus on site and were preserved in 90% ethanol that was later refreshed with 70% ethanol and stored at -20^{o}C in the laboratory. Of the larvae collected in each pond, I selected early instar larvae from the North Island complex and late instar larvae from the South Island complexes to ensure they were from similar cohorts as they begin their life-cycle at the beginning of the summer and require at least one year to complete larval development. Species identification was confirmed using taxonomic keys (Rowe 1987).

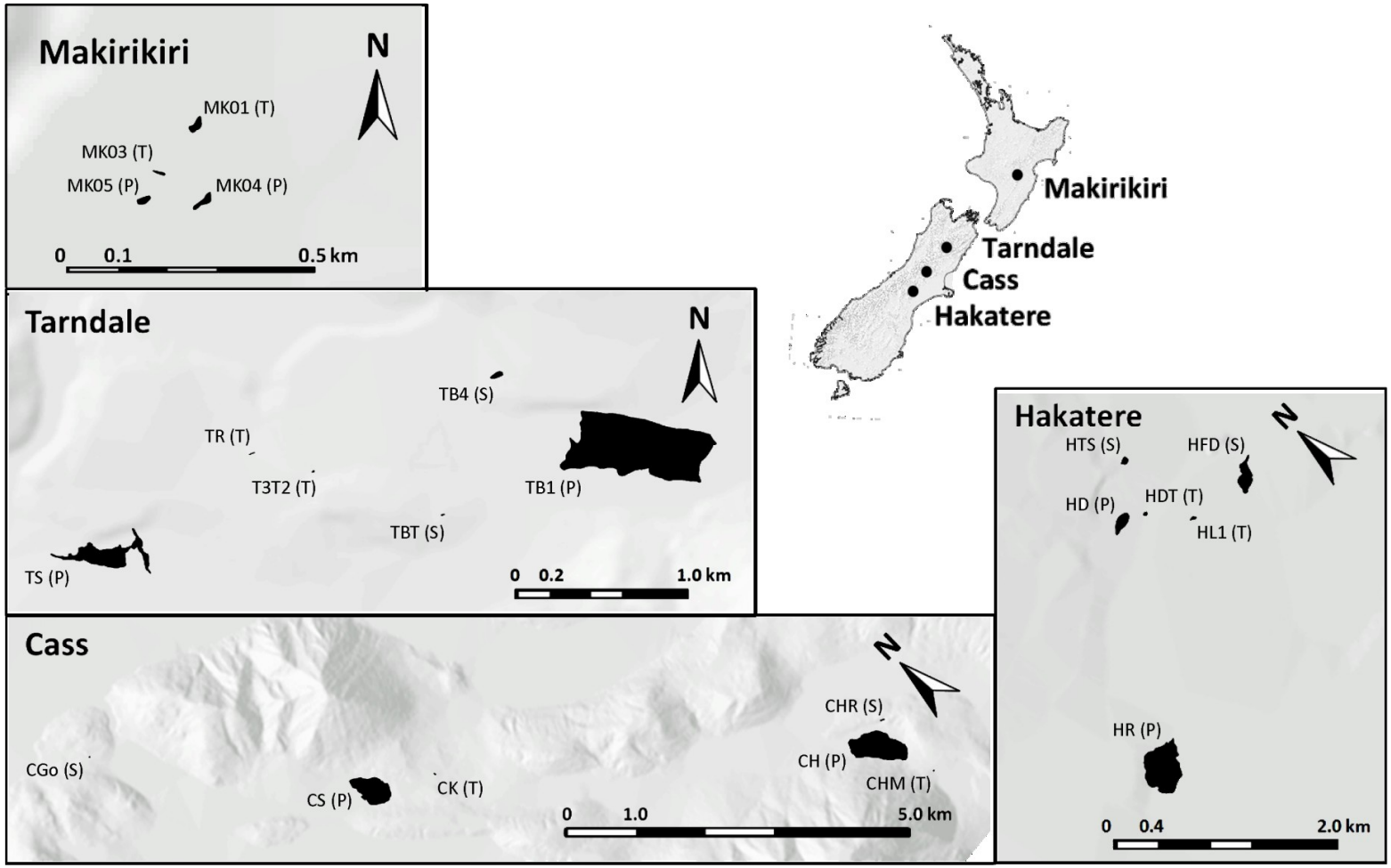

Figure 4.1. Four pond complexes in the North and South Islands, New Zealand where *Xanthocnemis zealandica* nymphs were collected for population genetics analyses. In each pond complex individual ponds are indicated by site abbreviations as in Table 4.1, with habitat type in parentheses (P: permanent, S: semi-permanent, T: temporary).

Table 4.1. Location and environmental characteristics of *Xanthocnemis zealandica* pond populations across four North and South Island, New Zealand, pond complexes. Collection sites were organised by island and pond complex, with site code, Universal Transverse Mercator coordinates, pond area, hydroperiod (temporary ponds experience annual drying, semi-permanent ponds that dry inter-annually, and permanent lakes never dry), the mean annual proportion each pond was full (i.e., mean wet), and the presence of predatory fish.

Island	Complex	Site	Site Code	UTM Northing	UTM Easting	Pond area (Ha)	Hydroperiod	Mean wet per annum	Fish
North	Makirikiri	Maki 04	MK04	2781100	6172071	0.04	Permanent	1.00	No
		Maki 05	MK05	2781094	6172051	0.05	Permanent	1.00	No
		Maki 01	MK01	2781208	6172206	0.04	Temporary	0.90	No
		Maki03	MK03	2781170	6172095	0.05	Temporary	0.87	No
South	Tarndale	Lake Bowscale	TB1	2505830	5897655	25.50	Permanent	1.00	Yes
		Lake Sedgemere	TS	2502860	5896785	6.63	Permanent	1.00	Yes
		Bowscale 4	TB4	2505330	5898065	0.19	Semi-permanent	1.00	No
		Bowscale Tarn	TBT	2505335	5897295	0.01	Semi-permanent	1.00	No
		Roadside	TR	2504000	5897510	0.02	Temporary	0.94	No
		3 Temp 2	T3T2	2504185	5897450	0.02	Temporary	0.57	No
	Cass	Lake Sarah	CS	2410135	5794680	20.00	Permanent	1.00	Yes
		Lake Hawdon	CH	2416380	5788975	30.00	Permanent	1.00	Yes
		Hawdon Ridge	CHR	2416795	5788880	0.34	Semi-permanent	1.00	No
		Goldney	CGo	2407660	5798715	0.09	Semi-permanent	0.99	No
		HM Depression	CHM	2416655	5787685	0.07	Temporary	0.74	No
		Kettlehole	CK	2410975	5794180	0.03	Temporary	0.67	No
	Hakatere	Lake Roundabout	HR	2356420	5729970	12.12	Permanent	1.00	Yes
		Lake Donne	HD	2357880	5731485	0.29	Permanent	1.00	Yes
		Fagan Downs	HFD	2358960	5730800	0.58	Semi-permanent	1.00	Yes
		Tiny Spider	HTS	2358370	5731820	0.11	Semi-permanent	1.00	No
		Donne Temp	HDT	2358015	5731415	0.04	Temporary	0.83	No
		Lambies 1	HL1	2358225	5730965	0.02	Temporary	0.89	No

DNA extraction and amplification

DNA was extracted following the Lopera-Barrero et al. (2008) NaCl-extraction protocol modified for *X. zealandica*. For each specimen, the heads were removed and eyes were cut off to retain pigment-free tissue. The labium was also cut from the head and stored with the rest of the body in 70% ethanol at -20^{o}C for confirmation of species identification. Extracted DNA was stored at -20^{o}C. I genotyped individuals at eight microsatellite loci using markers designed for *X. zealandica* (Table 4.S1). Multiplex PCR amplification of the microsatellite loci was done using a Qiagen Type-it® kit with 2 µL reaction volumes consisting of Type-it Multiplex PCR Master Mix, and three primers: 0.0162 - 0.0216 µm of forward primer with m13 tag, 0.0648 – 0.0864 µm of reverse primer (Table 4.S1), and 0.135 µm M13 5'-end labelled dye (VIC, NED, FAM, or PET), and 0.82 to 0.87 µL RNAse-free water (depending on multiplex and primers). This reaction mixture was added to 1 uL of template DNA diluted (1:10) to a final concentration of 4.2 – 35.6 ng·µL^{-1}. PCR reactions were: 15 min. at 95^{o}C, eight cycles of 30 sec. at 94^{o}C, 1:30 min. at 56^{o}C, and one min. at 72^{o}C; followed by 25 cycles of 30 sec. at 94^{o}C, 1:30 min at 52^{o}C, and one min. at 72^{o}C; and one cycle at 60^{o}C for 30 min. Post PCR products were diluted with 25 µL MilliQ H_2O. Final PCR products were genotyped by the University of Canterbury Sequence Service on an Applied Biosystems 3100 DNA analyser with GeneScan 500LIZ internal size standard (ABI). Alleles for each locus were scored using Gene Marker v.1.6 (SoftGenetics LLC). To ensure genotype accuracy for each PCR reaction we included at least three negative controls per extraction and amplified a known genotype in each reaction. Replicates (minimum 10% of sample size) were screened for amplification and scoring errors.

Standard genetic analyses

All loci were assessed for null alleles and genotyping errors using Microchecker v.2.2.3 (van Oosterhout et al. 2004). In addition, tests of linkage disequilibrium to assess pairwise independence of loci and deviation from Hardy-Weinberg equilibrium were run using Fisher's exact tests within each site (Arlequin v3.5.1.3; Excoffier and Lischer 2010, Slatkin and Excoffier 1996). Due to the large number of tests (n = 176), statistical significance (α = 0.05) was adjusted by a Dunn-Sidak correction ($1-(1-\alpha)^{1/n}$) to 0.000291. The mean number of alleles were calculated per locus (N_A), rarified allelic richness (A), and private alleles (A_P) across loci for each site using HP-RARE v. 1.1 (Leberg 2002, Kalinowski 2005) with the lowest sample size (n = 6). Observed (H_O) and unbiased expected (H_E) heterozygosities were estimated with GenAlEx v. 6.5 (Peakall and Smouse 2006). To evaluate differences in allelic diversity (A, A_P, and H_E), I used separate nested analyses of variance (ANOVA) with pond complex as the main effect and habitat-type nested within pond complex, followed by Tukey post-hoc tests using R version 3.0.2 (R Development Core Team 2013). To calculate pond-specific inbreeding coefficients (F_{IS}), I used Arlequin v. 3.5.1.3.

Population structuring

Through a series of hierarchical analyses of molecular variation (AMOVA, Balloux and Lugon-Moulin 2002), I tested potential genetic partitioning by island, among South Island pond complexes, and habitats within each pond complex using Arlequin v. 3.5.1.3 (10,000 permutations; loci with 95% CI bootstrapped 20,000 times). To determine whether there was differentiation between specific ponds due to spatial or habitat characteristics I calculated both pairwise G_{ST} and Jost's *D* using the R program DEMEtics (bootstrapping = 1000, Gerlach et al. 2010). In addition to the fixation index G_{ST} (Nei 1973), I used Jost's *D* because

it can perform better with polymorphic markers and uneven sample sizes (Jost 2008). DEMEitcs applied a modified Benjamini-Hochberg False Discovery Rate for multiple statistical tests to *p*-values for a family-wise error rate of $\alpha = 0.05$ (Benjamini and Yekutieli 2001, Narum 2006). To determine whether there was pairwise differentiation patterns with G_{ST} and Jost's *D*, I used separate Principle Coordinates Analyses (PCoA) in Genalex v.6.5. The resultant PCoA Eigen vectors were plotted for the two principle coordinates with the highest percent probability.

I also tested for isolation-by-distance in the South Island, and within each pond complex using a series of MANTEL tests in GenAlEx v. 6.5 (999 permutations) with two different geographic Euclidean distance matrices (distance in kilometres and log- transformed distance). A Bayesian genetic clustering program, STRUCTURE v. 2.3.4 (Prichard et al. 2000), was also used to distinguish potential populations by using multi-locus genotypes to assign individuals to populations based on their inferred allelic frequencies. For this program, I allowed for admixture with no *a priori* definitions of population identification. To investigate potential population assignments at various geographic scales, STRUCTURE was used to identify populations at three levels: the North/South Island, pond complexes within the South Island, and individual pond complexes. STRUCTURE was run with the following conditions: 10,000 burn-in, 100,000 Markov-chain Monte-Carlo (MCMC) iterations post burn-in, correlated allele frequencies, admixture model, inferred α with uniform prior, number of populations (*K*) = 1-10 with 10 replicates. From these analyses, I estimated the optimal genetic clusters (*K*) for STRUCTURE using the log-likelihood among *K* values and the change in log-likelihood values (ΔK) corrected by the variance among replicate runs (Evanno et al. 2005) with the program Structure Harvester web v. 0.6.93 (Earl and vonHoldt 2012).

Environmental predictors of genetic structure

To evaluate whether genetic divergence through pond specific F_{ST} was influenced by local environmental conditions I used GESTE v. 2.0 (Foll and Gaggiotti 2006, Gaggiotti and Foll 2010). This program utilises a hierarchical Bayesian test of the *F*-model to estimate population-specific F_{ST} from the observed genetic data with a likelihood function to determine allelic frequencies for each population and the F_{ST} values are then related to environmental factors with regression models using a lognormal prior. The environmental factors are used to explain variation in population-specific F_{ST} resulting from different rates of migration and drift in each population. Each model includes a constant term and parameters representing the effect of environmental factors on population-specific F_{ST} along with an error term that is estimated for each model. Each model set includes a constant, individual factors, and all possible environmental factor interactions. I evaluated the effects of five factors on specific pond F_{ST}: 1) fish presence, 2) hydroperiod, 3) pond size, 4) longitude, and 5) latitude, resulting in a model set with 32 model combinations. Fish presence in each pond was determined during aquatic sampling over a three-year (2010-2013) field survey (Chapter Two). Hydroperiod was measured as the mean annual proportion each pond was dry between 2010-2013. Small pond sizes were manually measured in the field, while larger ponds were measured from Google Earth v7.0.2 satellite images and confirmed against published records where available (Irwin 1975). To evaluate the influences of spatial distances between the ponds, latitude (Northing) and longitude (Easting) were included as separate factors as GESTE only allows single values for each population. GESTE v 2.0 uses a reversible jump MCMC to estimate model posterior means of population-specific F_{ST} and the posterior probability and parameters for each model. The model with the highest posterior probability is the most supported and overall support for individual environmental factors is evaluated through a sum of posterior probabilities across all models that include a given

factor. To test the model set, I ran GESTE with 10 pilot runs of 5000 iterations, 500,000 burn-in, a thinning interval of 50, and a 30000 sample size to obtain parameter estimates. To evaluate the role of environmental factors at all spatial scales, I repeated the model runs at the national scale, South Island pond complexes, and for each pond complex.

Analyses of potential gene flow/bottlenecks

To determine whether pond drying or predatory fish caused bottlenecks or source-sink dynamics in the temporary and permanent ponds I estimated contemporary dispersal between habitats and the potential for recent bottlenecks influencing the excess of heterozygotes in the populations. I used BAYESASS v 3.0.3 (Wilson and Rannala 2003) to estimate the proportion of recent migrants (*m* = migration rates) over the last several generations through a Bayesian assignment method. This method is based on the principle that immigrants and their offspring have temporary disequilibrium in their microsatellite genotypes but does not assume that populations are in migration-drift or Hardy-Weinberg equilibrium. Additionally, BAYESASS assumes immigrants constitute a maximum of 33% of a recipient population and loci are in linkage equilibrium. Because larger numbers of populations (> 7) can reduce the accuracy of identifying migrant sources (Faubet et al. 2007), I evaluated the proportion of migrants in separate analyses for long distance and local migration. By pooling the individuals by pond complex, I could evaluate long distance migration, while I separately tested for local migration among ponds within each pond complex. Default delta values for allele frequency, migration rate, and inbreeding were adjusted to ensure chain swapping occurred in 40-60% of the total iterations as recommended by Wilson and Rannala (2003). Once adjusted, analyses were run for $6x10^7$ iterations, MCMC burn-in of $1x10^7$, and sampled every 2000 iterations, with three replicates of each spatial scale performed using different

random seed numbers to ensure consistency among runs. To evaluate the convergence and consistency of posterior probability estimates among replicates, I used the program Tracer v 1.6 (Rambaut et al. 2013) to compare a scenario using default delta values only. Estimates of the proportion of unidirectional migrants were made for each pair of sites with 95% confidence intervals at the different spatial scales: national level and separately for each pond complex.

To detect recent bottlenecks, two tests were performed with BOTTLENECK v. 1.2.02 (Piry et al. 1999): the Wilcoxon's sign-rank test which is based on the excess of heterozygotes, and the mode-shift test that evaluates the allele frequency distribution. Evidence of bottlenecks are detectable over $< 4N_e$ generations using heterozygote excess and a few dozen generations with mode-shift test (Corneut and Luikart 1996). Both tests were performed using the stepwise mutation model (SMM) and the two-phase model (TPM) which are recommended by Corneut and Luikart (1996). The Wilcoxon's sign-rank test with 95% SMM was used as a conservative setting for bottleneck detection as suggested by Williamson-Natesan (2005). TPM was run with 95% single-step mutations and 5% multi-step mutations with a variance of 12 for mutation size as suggested by Piry et al. (1999) over 100,000 simulations. The mutation models have shown strong effects on the estimation of heterozygote excess, but do not distort the allele frequency as rare alleles are expected to be abundant regardless of the mutation model (Nei et al. 1976). These tests for bottlenecking were performed for all ponds in the North and South Island and again with ponds pooled into the four pond complexes as the bottlenecking may be acting on a pond complex level rather than at the individual pond level since temporary pond populations may be completely extinguished during long dry periods and subsequently recolonised from the permanent ponds.

Results

Standard genetic analyses

All eight loci were polymorphic with a total of 10 (XZ19 and XZ23) to 24 (XZ04) alleles (Table 4.S1). Significant deviation from HWE was observed in all but two loci (XZ17 and XZ23) after applying a Dunn-Sidak correction for multiple tests ($\alpha = 0.000291$, Table 4.S2). However, of the loci with deviations from HWE, significant tests were found in a small number of sites, with the exception of locus XZ19 that deviated from HWE in eight of 22 sites. In addition, XZ19 had evidence of null alleles in all 22 sites. Analyses of most parameters, including and excluding XZ19, were not significantly affected, except the measure of F_{IS}. Therefore, to limit the influence of these deviations, locus XZ19 was not included in subsequent analyses. There was little evidence of linkage disequilibrium for combinations among loci at each site with the exception of two sites in Tarndale pond complex (Lake Bowscale: loci combinations XZ14 and XZ11; XZ03, and Roadside XZ10 and XZ23). Because these loci pairs were not consistent across multiple sites physical chromosomal linkage is unlikely and the markers were considered independent replicates of the *X. zealandica* genome.

The final set of seven loci was successfully amplified for a total of 473 larvae from 22 populations distributed over the North and South Islands of New Zealand. Overall, allelic richness (2.93 ± 0.06) and heterozygosity were high ($H_O = 0.54 \pm 0.02$, $H_E = 0.61 \pm 0.02$), with an overall excess of homozygotes, and many populations had a significant effect of inbreeding (Table 4.2). The North Island were less diverse ($A_R = 2.82 \pm 0.14$) than the South Island ($A_R = 2.95 \pm 0.07$), and while the North Island observed and expected heterozygosity were not significantly different ($H_O = 0.60 \pm 0.04$, $H_E = 0.59 \pm 0.04$), the South Island had lower observed than expected heterozygosity ($H_O = 0.53 \pm 0.02$, $H_E = 0.61 \pm 0.02$; Table 4.2).

Table 4.2. Estimates of genetic diversity for *Xanthocnemis zealandica* populations and their associated pond complex with the number of larvae (N), number of alleles (A), rarefied allelic richness (A_R), and rarified private alleles (A_P). Observed (H_O) and expected (H_E) heterozygosities, and inbreeding coefficient (F_{IS}). All results are ± standard error (SE), *** > 0.001, ** > 0.01, * > 0.05, ns < 0.05.

Site	Habitat	N	A		A_R		A_P		H_O		Unbiased H_E		F_{IS}
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean
North Island		94	5.14	0.33	2.82	0.14	0.11	0.02	0.60	0.04	0.59	0.04	-0.0452 ns
Makirikiri													
Maki 04	Permanent	18	4.71	0.68	2.71	0.31	0.10	0.05	0.54	0.09	0.55	0.09	0.0260 ns
Maki 05	Permanent	30	5.57	0.69	2.72	0.28	0.12	0.04	0.60	0.09	0.57	0.08	-0.0440 ns
Maki 01	Temporary	26	4.86	0.59	2.94	0.29	0.11	0.04	0.58	0.08	0.62	0.09	0.0034 ns
Maki 03	Temporary	20	5.43	0.75	2.89	0.31	0.11	0.03	0.66	0.08	0.61	0.08	-0.1848 ns
South Island		379	5.78	0.18	2.95	0.07	0.11	0.01	0.53	0.02	0.61	0.02	0.0825***
Tarndale		125	5.36	0.32	2.83	0.12	0.12	0.02	0.49	0.03	0.58	0.03	0.0794***
Lake Bowscale	Permanent	9	4.14	0.63	2.92	0.35	0.09	0.06	0.53	0.11	0.59	0.09	0.0805 ns
Lake Sedgemere	Permanent	30	6.00	0.58	2.92	0.36	0.08	0.05	0.52	0.10	0.60	0.09	-0.0002 ns
Bowscale 4	Semi-permanent	21	6.29	0.97	3.01	0.33	0.10	0.04	0.50	0.07	0.62	0.08	0.1269*
Bowscale Tarn	Semi-permanent	15	4.71	0.42	2.74	0.23	0.12	0.06	0.44	0.06	0.58	0.07	0.2052*
Roadside	Temporary	20	4.57	0.78	2.62	0.32	0.15	0.05	0.43	0.08	0.53	0.08	0.0937 ns
3 Temp 2	Temporary	30	6.43	0.92	2.78	0.31	0.18	0.06	0.54	0.06	0.56	0.08	0.0387 ns
Cass		135	6.29	0.33	3.04	0.12	0.12	0.02	0.57	0.03	0.62	0.03	0.0563**
Lake Sarah	Permanent	28	6.29	0.78	3.16	0.26	0.09	0.05	0.62	0.08	0.67	0.06	0.0595 ns
Lake Hawdon	Permanent	12	5.14	0.80	3.04	0.35	0.13	0.05	0.50	0.08	0.63	0.08	0.2319**
Hawdon Ridge	Semi-permanent	18	6.29	0.99	3.03	0.31	0.22	0.09	0.59	0.10	0.63	0.07	-0.0770 ns
Goldney	Semi-permanent	24	6.71	0.81	3.01	0.36	0.09	0.03	0.58	0.08	0.60	0.09	0.0307 ns
HM Dep	Temporary	27	6.71	0.87	3.10	0.33	0.09	0.03	0.60	0.10	0.64	0.09	0.0184 ns
Kettlehole	Temporary	26	6.57	0.72	2.90	0.30	0.12	0.04	0.52	0.06	0.58	0.08	0.0757 ns
Hakatere		119	5.69	0.28	3.00	0.11	0.09	0.01	0.53	0.04	0.63	0.02	0.0969***
Lake Roundabout	Permanent	24	5.29	0.64	2.74	0.26	0.06	0.02	0.50	0.11	0.58	0.06	0.0012 ns
Lake Donne	Permanent	24	6.57	0.72	3.14	0.30	0.10	0.05	0.59	0.09	0.65	0.07	0.0238 ns
Fagan Downs	Semi-permanent	29	6.29	0.81	3.09	0.31	0.08	0.03	0.58	0.09	0.65	0.07	0.1273**
Tiny Spider	Semi-permanent	13	6.00	0.62	3.24	0.24	0.12	0.04	0.51	0.09	0.69	0.04	0.0909 ns
Donne Temp	Temporary	16	5.43	0.48	2.95	0.25	0.12	0.05	0.48	0.10	0.61	0.06	0.1073 ns
Lambies 1	Temporary	13	4.57	0.69	2.82	0.23	0.07	0.03	0.54	0.09	0.62	0.05	0.2537**
Overall		473	5.66	0.16	2.93	0.06	0.11	0.01	0.54	0.02	0.61	0.02	0.0922 ns

According to the nested ANOVAs the main effects of pond complex were significant for allelic richness, expected heterozygosity, and difference in observed and expected heterozygosity (Figure 4.2). Allelic richness was highest in the two southern pond complexes, Cass and Hakatere ($F_{3,11} = 4.34$, $p = 0.03$), with significant differences between one of the southern pond complexes, Cass, and the northern pond complexes, Makirikiri ($p = 0.04$) and Tarndale ($p = 0.03$; Figure 4.2a). A similar pattern was found in the expected heterozygosity with greater genetic diversity further south ($F_{3, 11} = 5.56$, $p = 0.014$), with Cass ($p = 0.05$) and Hakatere ($p = 0.02$) significantly different from the Tarndale pond complex (Figure 4.2c). In addition, the differences between observed and expected heterozygosity were significantly higher for Makirikiri ($F_{3,11} = 6.37$, $p = 0.009$) than both Tarndale ($p = 0.01$) and Hakatere ($p = 0.01$). Makirikiri was the only pond complex without a higher expected than observed heterozygosity (Figure 4.2d). However, there were no significant differences in the mean number of private alleles among pond complexes ($F_{3,11} = 0.99$, $p = 0.43$; Figure 4.2b), nor in nested effect of habitat type for the allelic richness ($F_{7,11} = 1.71$, $p = 0.20$), private alleles ($F_{7,11} = 1.19$, $p = 0.38$), expected heterozygosity ($F_{7,11} = 2.52$, $p = 0.08$), or difference in observed versus expected heterozygosity ($F_{7,11} = 0.84$, $p = 0.57$).

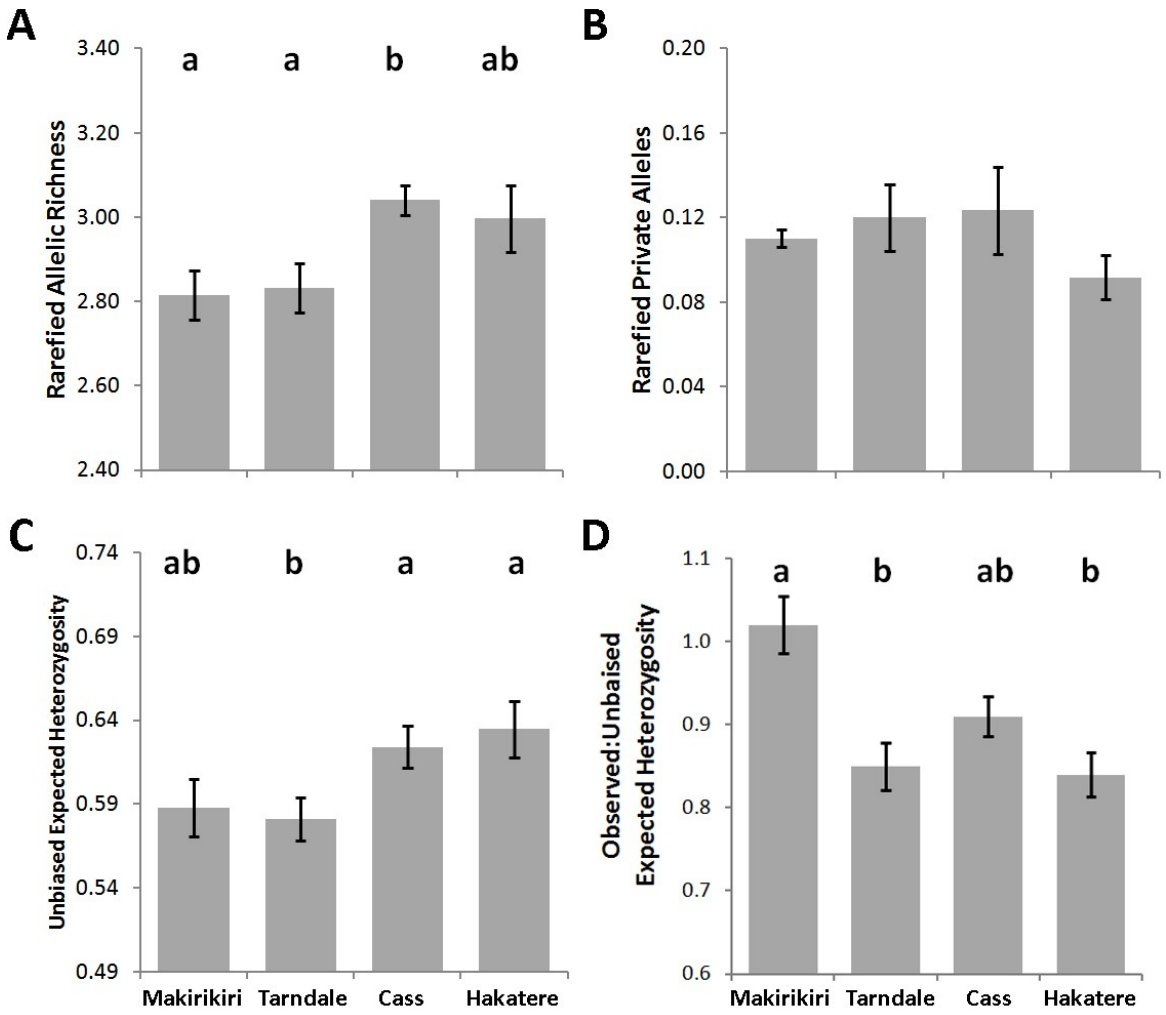

Figure 4.2. Mean allelic richness (A), private alleles (B), expected heterozygosity (C) and difference in observed versus expected heterozygosity (D) among the four pond complexes. Lower-case letters indicate significant differences (± S.E.). There were no significant within effects (habitat type).

Population structuring

Analyses of the different hierarchical spatial scales found significant genetic differentiation at all levels, with the highest variation between the North and South Islands (F_{ST} = 0.125, p < 0.001). F_{ST} values were low but significantly different among the South Island pond complexes (F_{ST} = 0.013, p < 0.001) and at the individual pond complexes (F_{ST} = 0.012 – 0.026, p = 0.01 – 0.001), with the exception of the Makirikiri pond complex (F_{ST} = 0.013, p = 0.312; Table 4.3). When the South Island ponds were analyzed based on their habitat type, rather than by pond complex, there was very little, but significant, differentiation (F_{ST} = 0.007, p < 0.001), with weak population structuring between different habitat types (Table 4.3). However, across all scales analyzed, the most significant variation (p < 0.001) was found within individuals and populations, with the exception of the North Island which had no significant F_{ST}, F_{IS}, or F_{IT} values. Population-specific F_{IS} estimates from Arlequin ranged from 0 to 0.25, with significant inbreeding coefficients only present in the South Island. In the North Island, there was no evidence of inbreeding across all ponds. The five South Island ponds (Tarndale: Bowscale 4 and Bowscale Tarn, Cass: Lake Hawdon, and Hakatere: Fagan Downs and Lambies 1) with significant inbreeding were not especially spatially isolated and inbreeding occurred across all habitat types (Table 4.2).

Table 4.3. Global *F*-statistics (F_{ST}, F_{IS}, F_{IT}) with associated 95% confidence intervals and percentages of explained variation for *Xanthocnemis zealandica* larvae at three hierarchical spatial scales and analyzed for variation in pond complex or habitat type. α > 0.001***, > 0.01**, > 0.05*, < 0.05 ns.

Spatial scale		F_{ST}	F_{IS}	F_{IT}
North and South Island	F-statistic	0.1249***	0.1194***	0.2294***
	(95% CI)	(0.04533-0.21439)	(0.07679-0.16325)	(0.13994-0.31918)
	Variation (%)	12.4879	10.44963	77.06246
South Island only	F-statistic	0.0127***	0.1414***	0.1523***
(Pond complexes)	(95% CI)	(0.00929-0.01683)	(0.08061-0.19908)	(0.09235-0.20954)
	Variation (%)	1.2698	13.95632	84.7739
South Island only	F-statistic	0.0069***	0.1447***	0.1506***
(Habitat types)	(95% CI)	(0.00036-0.01495)	(0.08554-0.20267)	(0.09113-0.20935)
	Variation (%)	0.6900	14.37055	84.9395
Pond complexes:				
Makirikiri	F-statistic	0.0132 ns	-0.0159 ns	-0.0026 ns
(Habitat types)	(95% CI)	(-0.00215-0.03614)	(-0.10812-0.06238)	(-0.09896-0.08047)
	Variation (%)	1.3152	-1.5716	100.2565
Tarndale	F-statistic	0.0255***	0.1593***	0.1808***
(Habitat types)	(95% CI)	(-0.00103-0.05045)	(0.09024-0.22310)	(0.09916-0.26419)
	Variation (%)	2.5529	15.5236	81.9235
Cass	F-statistic	0.0119*	0.0847***	0.0956***
(Habitat types)	(95% CI)	(0.00575-0.01849)	(0.04659-0.12732)	(0.05586-0.13887)
	Variation (%)	1.1863	8.3701	90.4437
Hakatere	F-statistic	0.0172*	0.1598***	0.1743***
(Habitat types)	(95% CI)	(0.00367-0.03161)	(0.04978-0.27173)	(0.06671-0.28471)
	Variation (%)	1.7205	15.7052	82.5743

Genetic differentiation between the North and South Island populations was reflected in the pairwise distance measures, G_{ST} and Jost's *D*, where the North Island pond populations are significantly different from all the South Island populations. Among the South Island populations, three sites (Tarndale: Bowscale Tarn and Hakatere: Lambies 1) were significantly different from all others in the study for both G_{ST} and Jost's *D* (Table 4.S3). Principle coordinate analyses of these pairwise measures indicated differentiation between the North and South Island populations along the first coordinate axis which accounted for 59.0% (G_{ST}) and 50.1% (Jost's *D*) of the microsatellite variability, but there was overlap among habitat types with no further structure evident along the second principle coordinate (G_{ST} = 17.0%, Jost's *D* = 18.9%, Figure 4.3). Restricting the analyses to the South Island revealed the two ponds with strong genetic differentiation, without these ponds there were temporary and permanent pond clusters with overlap by semi-permanent ponds (principle coordinate axis variation G_{ST}: 36.7% and 25.4%; Jost's *D*: 30.8% and 23.0%). In addition, there were separate clusters between Tarndale (northern) and Hakatere (southern) pond complexes along the second axes with overlap by the Cass ponds (Figure 4.3).

Bayesian clustering incorporating all individuals suggested distinct North Island and South Island population clusters ($\Delta K = 2$, Figure 4.4a). When the two islands were analyzed separately there were minor differences in assignment frequencies between the temporary and permanent ponds in the North Island. Further analysis resolved the variability in South Island population clusters ($\Delta K = 4$) with two populations distinct from all others, Bowscale Tarn (Tarndale) and Lambies 1 (Hakatere) (Figure 4.4b). Aside from these two ponds there were no obvious clustering patterns due to habitat type (Figure 4.4c). While not statistically significant, MANTEL tests revealed a trend for spatial variation among the South Island ponds for Euclidean distance ($r_{xy} = 0.14$, $p = 0.054$; Table 4.S4). Within each pond complex there was no evidence of isolation-by-distance (Table 4.S4).

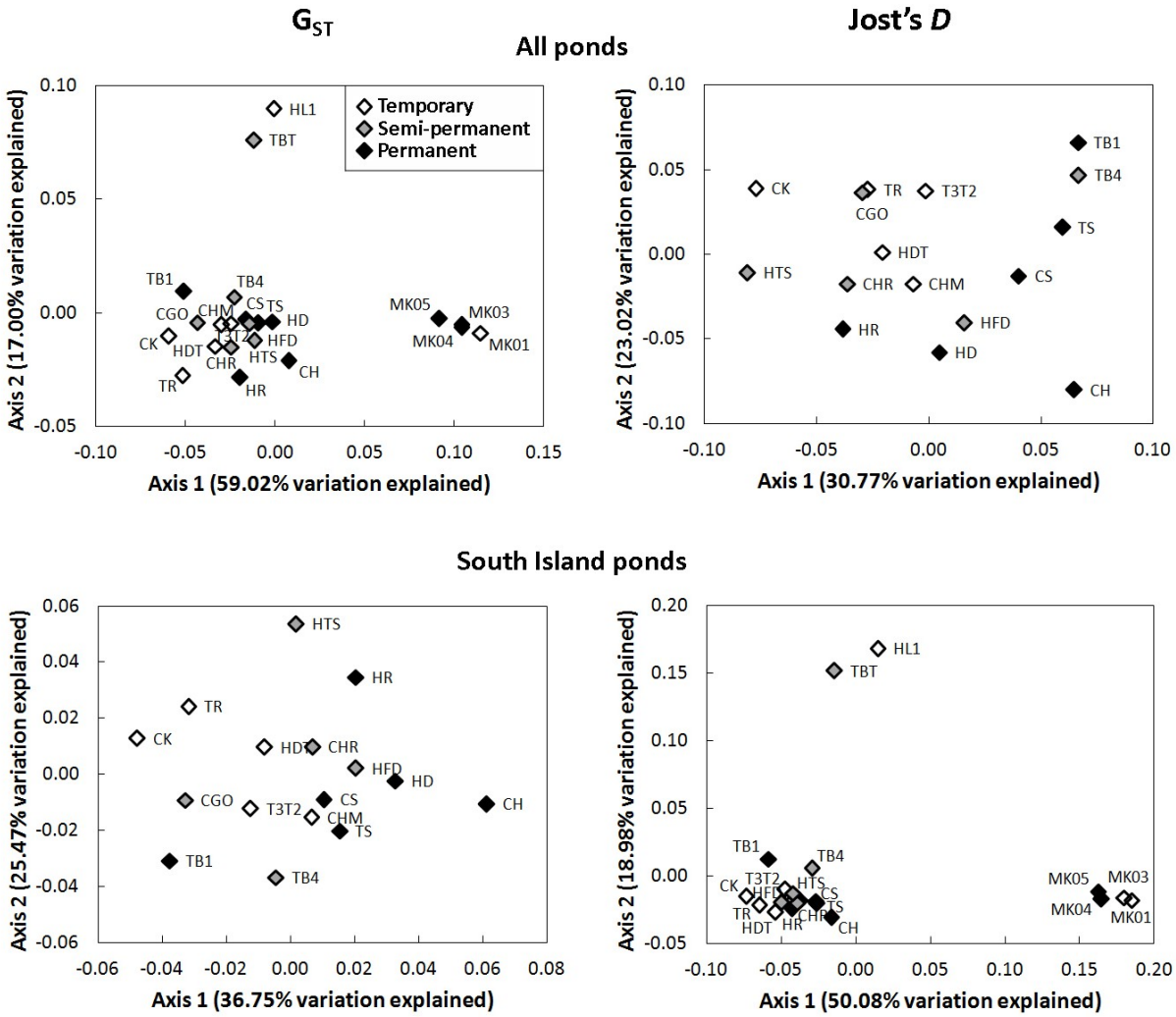

Figure 4.3. Principal coordinates analyses on bi-dimensional dispersion of G_{st} and Jost's *D* using *Xanthocnemis zealandica* loci for all ponds and only South Island pond. Icon colours represent the different habitat types (permanent: black, semi-permanent: grey, temporary: white) and their associated pond names. Ponds starting with C (Cass), H (Hakatere), T (Tarndale), and M (Makirikiri) denote the associated pond complexes. South Island pond analyses do not include two outlier pond populations (Hakatere: Lambies 1 and Tarndale: Bowscale Tarn).

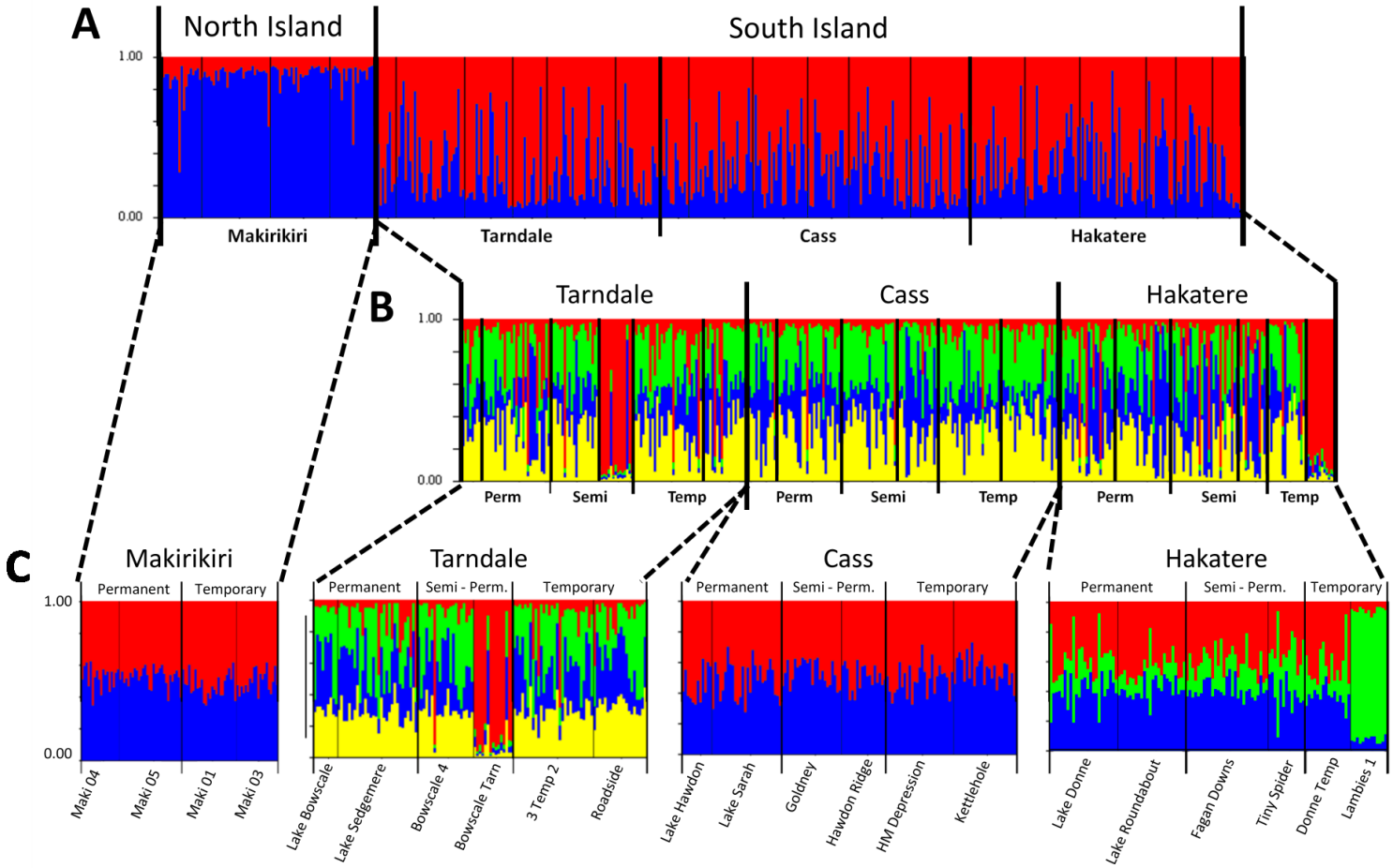

Figure 4.4. Population structure in *Xanthocnemis zealandica* at three hierarchical spatial scales inferred from Bayesian clustering analysis using separate STRUCTURE analyses for (A) North and South Island, (B) South Island, and (C) each pond complex, with individual admixture proportions for different clusters (K) labelled in separate colours.

Environmental predictors of genetic structure

Across all pond populations the model of genetic divergence with the highest posterior probability included effects of latitude and longitude (Table 4.4). Local F_{ST} was positively related to latitude ($r^2 = 0.49$) and longitude ($r^2 = 0.48$). Relative to spatial divergence the other environmental characteristics (pond area, hydroperiod, and fish presence) were poor predictors of genetic structuring at all hierarchical levels. Restricting analyses to the South Island and specific pond complexes showed all environmental predictors, including latitude and longitude, had low posterior probabilities (< 0.067) compared to the null model (Tables 4.4 and 4.5).

Table 4.4. Posterior probabilities for the eight most-supported environmental models of genetic divergence (local F_{ST}) of *Xanthocnemis zealandica* North and South Island populations and sum of posterior probabilities of each model with a given factor.

Most probable models

North and South Island

Model	Posterior probability
Latitude	0.268
Longitude	0.259
Null	0.167
Latitude + Longitude	0.158
Fish	0.018
Latitude + Fish	0.014
Latitude + Hydrology	0.013
Longitude + Hydrology	0.013

South Island

Model	Posterior probability
Null	0.665
Latitude	0.068
Longitude	0.062
Fish	0.043
Hydrology	0.040
Latitude + Longitude	0.038
Habitat area	0.037
Fish + Habitat area	0.006

Sum of posterior probabilities

Factor	Sum
Latitude	0.494
Longitude	0.482
Fish pres/abs	0.064
Hydrology	0.049
Habitat area	0.048

Factor	Sum
Latitude	0.126
Longitude	0.121
Fish pres/abs	0.065
Hydrology	0.059
Habitat area	0.058

Table 4.5. Posterior probabilities for the eight most-supported environmental models of genetic divergence (local F_{ST}) of *Xanthocnemis zealandica* populations within each pond complex and sum of posterior probabilities of each model with a given factor.

Most probable models

Makirikiri

Model	Posterior probability
Null	0.5800
Longitude	0.0781
Habitat area	0.0768
Hydrology	0.0760
Latitude	0.0744

Tarndale

Model	Posterior probability
Null	0.596
Latitude	0.064
Hydrology	0.064
Habitat area	0.063
Fish	0.059
Longitude	0.059
Habitat area + Fish	0.013
Latitude + Longitude	0.011

Cass

Model	Posterior probability
Null	0.586
Habitat area	0.060
Latitude	0.058
Longitude	0.058
Fish	0.056
Hydrology	0.054
Latitude + Longitude	0.028
Habitat area + Fish	0.023

Hakatere

Model	Posterior probability
Null	0.547
Hydrology	0.069
Longitude	0.068
Fish	0.065
Latitude	0.062
Habitat area	0.056
Longitude + Habitat area	0.019
Longitude + Fish	0.013

Sum of posterior probabilities

Factor	Sum
Latitude	0.150
Longitude	0.146
Hydrology	0.139
Habitat Area	0.124

Factor	Sum
Habitat area	0.108
Fish pres/abs	0.106
Latitude	0.105
Hydrology	0.099
Longitude	0.098

Factor	Sum
Latitude	0.124
Longitude	0.121
Habitat area	0.120
Fish pres/abs	0.115
Hydrology	0.092

Factor	Sum
Longitude	0.137
Habitat area	0.122
Hydrology	0.121
Fish pres/abs	0.120
Latitude	0.113

Analyses of potential gene flow/bottlenecks

There were significantly asymmetric (non-overlapping 95% C.I.) estimates of recent long distance migration across the South Island and local migration among ponds within all four pond complexes. Migration rates ranged from low (0.009) to high (0.282) at both spatial scales. There was no evidence of recent gene flow between the North and South Islands, but within the South Island there was high gene flow from the Cass pond complex to both Tarndale and Hakatere pond complexes (Figure 4.5, Table 4.S5a). In addition, there was low, but significant, gene flow from Tarndale to Hakatere.

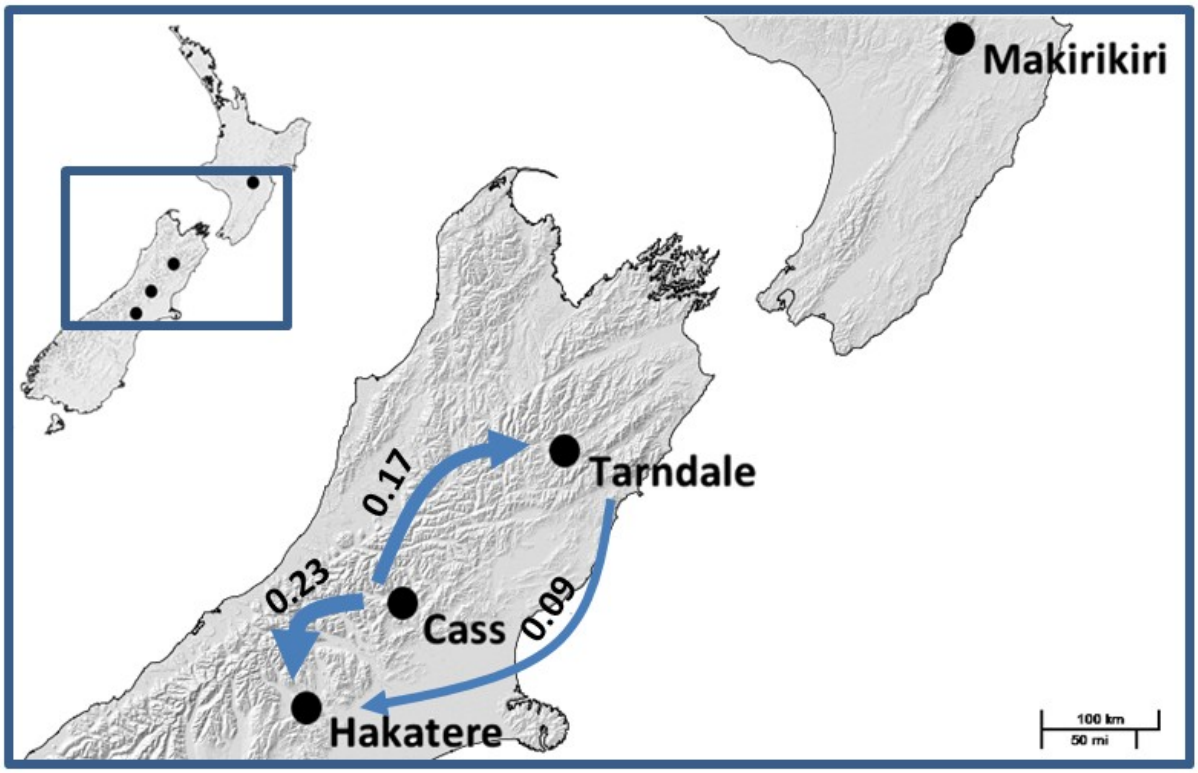

Figure 4.5. Estimates of contemporary gene flow with the proportion of immigrants across the four sampled pond complexes based on unidirectional assignments of microsatellite genotypes in BAYESASS v.3.0.3. The significant estimates of the proportion of immigrants and their population sources are indicated by arrows.

Local migration within the pond complexes generally had high gene flow from permanent ponds. In the Makirikiri pond complex, Maki 05 (permanent) was the only significant source of gene flow to all the other ponds. (Figure 4.6, Table 4.S5b). Lake Sedgemere (permanent) was the putative source population for all other Tarndale ponds except for Bowscale Tarn (semi-permanent) which had little evidence of immigration (Figure 4.6, Table 4.S5c). Within the Hakatere pond complex, the permanent Lake Roundabout significantly contributed migrants to all other ponds aside from temporary pond Lambies 1 (Figure 4.6, Table 4.S5e). While in the Cass pond complex, the temporary pond Kettlehole was the sole significant source of migrants to all ponds excluding Lake Hawdon (Figure 4.6, Table 4.S5d).

Overall, the gene flow patterns suggest a permanent source pond contributes the majority of migrants within each pond complex. However, the migration was not uniform across the pond complexes, as indicated by the lack of gene flow for Bowscale Tarn and Lambies 1, which supports the genetic differentiation found in the PCoA and STRUCTURE analyses (Figures 4.3 and 4.4b). Temporary ponds may also be important migrant sources as in the Cass pond complex. Interestingly, the gene flow results were uni-directional which may reflect general environmental factors (e.g., predominant wind direction), but this requires further investigation.

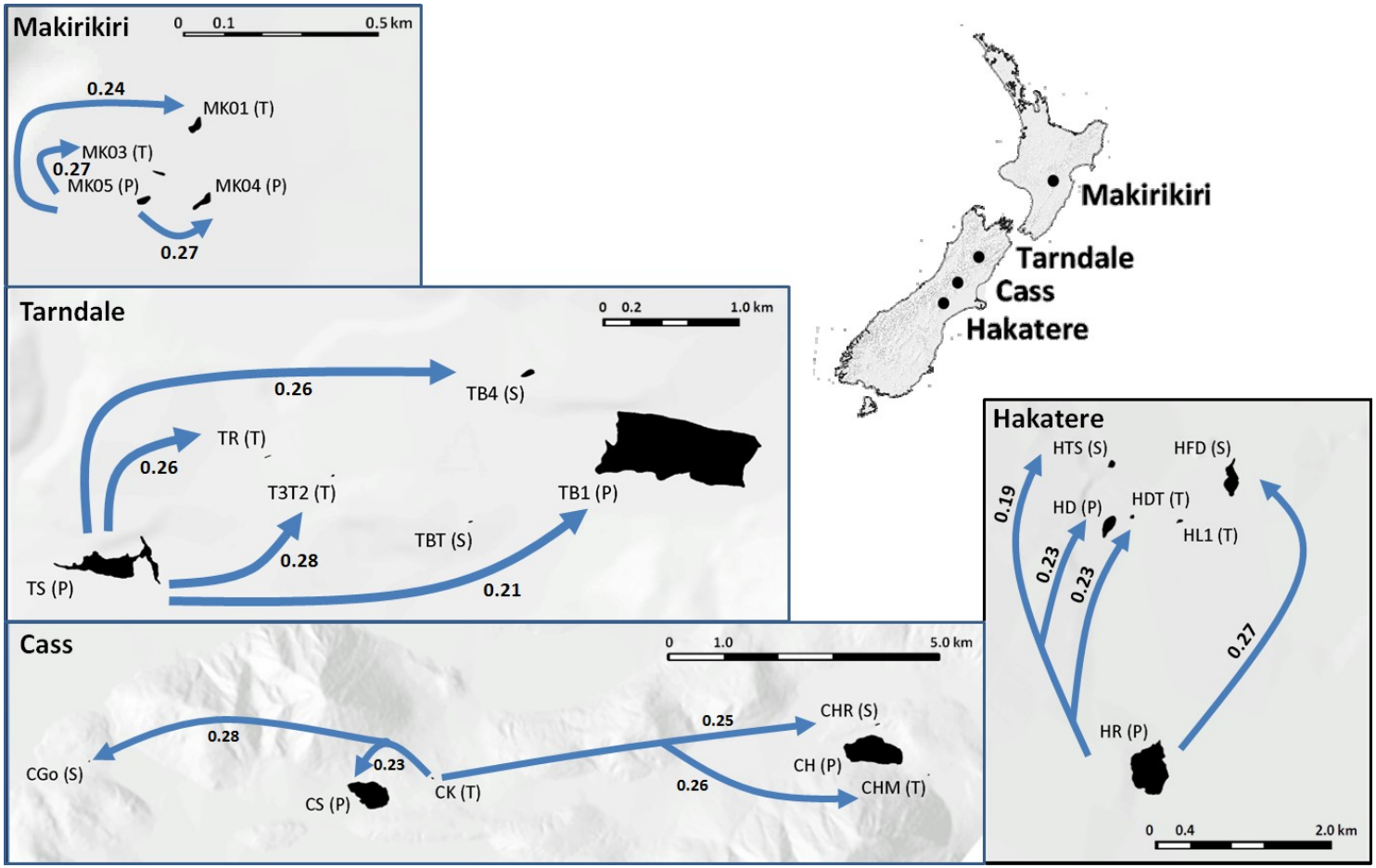

Figure 4.6. Estimates of contemporary gene flow with the proportion of immigrants for each pond complex based on unidirectional assignments of microsatellite genotypes in BAYESASS v.3.0.3. The significant estimates of the proportion of immigrants and their population sources are indicated by arrows. The pond abbreviation and habitat type, (P: permanent, S: semi-permanent, and T: temporary) are indicated for each pond.

Using the pure stepwise mutation model (SMM) and the two-phase mutation model (TPM; 95%SMM and 5% IAM) analyzed with Wilcoxon's tests, I did not detect recent bottlenecking footprints in any of the 22 pond populations or when the ponds were grouped into the four pond complexes. All but one permanent pond population (Tarndale: Bowscale Lake) had an L-shaped mode-shift that was expected under standard mutation-drift equilibrium with no evidence of recent bottlenecks. At the pond complex level there were no distortions in the L-shaped mode (Table 4.S6). Despite inter-annual variability in pond hydrology there was little evidence these unpredictable environmental conditions have caused recent bottlenecks within individual pond or across pond complex *X. zealandica* populations.

Discussion

Generalists that persist across a wide range of habitats may rely on dispersal between habitat types to counter multiple abiotic and biotic stresses that influence population stability. Gene flow between subpopulations that experience local extinctions and re-colonisations can result in metapopulation dynamics (Hanski et al. 2011, Huey et al. 2011). I found evidence for *X. zealandica* metapopulation dynamics across pond-permanence gradients with gene flow and limited divergence among different habitats within and among pond complexes throughout New Zealand. As expected, Cook Strait was an effective barrier resulting in genetic structuring between the North and South Islands. This contrasts with Nolan et al. (2007) who found *X. zealandica* homogeneous population structure across river catchments in the North and South islands using allozyme loci and mitochondrial DNA (mtDNA). Differences in our results can be attributed to the types of markers in each study. Allozyme and mtDNA analyses measure longer historic phylogenetic differences between populations, whereas microsatellite markers are typically a better measure of contemporary population structure

and gene flow (Wang 2010). Therefore, *X. zealandica* may experience low gene flow between the islands but did not have phylogenetically distinct populations.

I found differentiation between the islands, but at the lower hierarchical spatial scales there were uneven differences in population structuring. The North Island pond complex (Makirikiri) might be considered a null hypothesis with little evidence of population structure and relatively consistent genetic diversity among the habitat types. Because Makirikiri ponds were more tightly clustered, similar sized habitat types, and had no predatory fish, *X. zealandica* likely experiences few barriers to dispersal and readily mixed among ponds. In contrast, the South Island pond complexes were more heterogeneous, resulting in more multifaceted population structuring. Contrary to my predictions, the mountains and distance between the South Island pond complexes were not likely barriers to *X. zealandica* dispersal.

There were differences between the islands, but there was no evidence of isolation-by-distance and the limited South Island population structuring with low F_{ST} and limited Bayesian clustering suggests that adult *X. zealandica* effectively disperse among pond complexes and across multiple habitat types. These results suggest that individual pond complexes are not independent metapopulations and may exchange individuals by long-distance dispersers. However, there still may be larger geographic influences with increased allelic richness and expected heterozygosity in the southern pond complexes. Genetic distance (G_{ST} and Jost *D*) clusters in the South Island further support a north-south gradient. One possible explanation for the higher diversity in the southern pond complexes could be higher connectivity between neighbouring pond complexes, and Tarndale's higher elevation limited genetic exchange.

Within the South Island pond complexes there was evidence of potential isolation and uneven gene flow unrelated to general habitat characteristics. Three ponds stood out in the

STRUCTURE and PCoA analyses: Tarndale – Bowscale Tarn, Cass – Lake Hawdon, and Hakatere – Lambies 1, representing the different pond types (semi-permanent, permanent, and temporary, respectively). These ponds were not particularly isolated spatially within their pond complexes, but did experience lower gene flow than their complex neighbours (Figure 4.5). Additionally, these ponds did not have unique genetic diversity within their pond complexes, but had the highest levels of inbreeding (F_{IS}). The specific cause of the inbreeding and differentiation of these three populations remains unknown and could be that these ponds experience localised selection pressures, low immigration, or there are particularly high territorial behaviours in these ponds. Other studies of odonates have found aggressive territorial behaviour and affinity to natal habitats can greatly limit their dispersal distances despite their potentially high flight capability (McPeek 1989). While there were particular ponds within the South Island that had more distinct populations, there was also evidence of weak population structuring among the habitat types from F-tests and genetic distance measures, but not Bayesian tests. These results suggest that local environmental characteristics may influence *X. zealandica* population structure within heterogeneous pond complexes.

Environmental predictors of genetic structure

Generalist species can persist across heterogeneous habitats despite experiencing a range of environmental selection pressures that could influence their genetic diversity and population differentiation. Within the pond complexes, *X. zealandica* were exposed to a variety of selection pressures such as unpredictable inter- and intra-annual drying in temporary ponds and predatory fish in permanent ponds. Temporary habitats commonly have lower genetic diversity within populations and depending on the frequency and duration of disturbance can

lead to greater divergence among habitats due to stochastic founder effects, bottlenecks, and genetic drift associated with recolonisation (Shama et al. 2011). These trends are typically observed in species with low dispersal capacity or experience population isolation from disturbances (Shama et al. 2011, Zickovich and Bohonak 2007). Despite variability in pond environmental conditions across the pond complexes, habitat type had little influence on *X. zealandica* genetic diversity. However, there was mixed evidence that population structure may be influenced by habitat type. Low, but significant, F_{ST} values from pond complex AMOVAs (0.016 – 0.045) along with permanent and temporary pond clustering from genetic distance measures (G_{ST} and Jost *D*) imply there are environmental influences of population structuring at the pond complex level. Low F_{ST} values suggest that, in sampling gametes, there would be a low probability that they were identical by descent within the sampled ponds. Therefore, F_{ST} could not easily distinguish between populations. Moreover, there was overall similar genetic diversity across all the sampled ponds, but some populations had more homozygotes and significant inbreeding. This suggests there is a minor level of relatedness within the populations and the lack of heterozygosity might have caused these trends toward fixation. Furthermore, there were no distinct populations by habitat type in the Bayesian analyses and specific pond characteristics (hydroperiod, pond size, fish presence) were poor predictors of *X. zealandica* genetic divergence (local F_{ST}). In similar North American and European pond systems, hydroperiod and presence of predatory fish were weak predictors of genetic divergence in amphibian and aquatic invertebrate metapopulations due to connectivity between habitat types (De Block et al. 2005, Cosentino et al. 2012). This suggests these environmental factors might be weak selection pressures for a generalist species with strong dispersal capability.

Dispersal capability and flexibility to environmental conditions can influence the susceptibility of organisms to selection pressures across heterogeneous environments

(Bohonak and Jenkins 2003, McCauley 2007). Several models argue that generalist species should only be retained in heterogeneous environments with weak selection pressures, otherwise stronger environmental influences should drive local adaptations leading to greater genetic divergence (de Meeus and Goudet 2000, Debarre and Gandon 2011, Massol 2013). Studies of New Zealand freshwater invertebrate communities have found high proportions of generalist species in temporary habitats and have attributed their widespread distributions to highly unpredictable hydrology, extensive dispersal and oviposition among different habitat types, and weak predation pressures in permanent habitats (Winterbourn et al. 1981, Wissinger et al. 2006 and 2009, Storey and Quinn 2010, Greig et al. 2013).

There is evidence that *X. zealandica* has flexible juvenile development with annual generations in temporary ponds, and multi-year generations in more permanent ponds (Chapter Two). *X. zealandica* also has flexible anti-predator behavioural responses, with permanent pond nymphs better able to avoid predatory fish through their slower movements and refuge-use (Chapter Three). Additionally, under experimental conditions *X. zealandica* nymphs tolerated short desiccation periods (< eight days without water) regardless of their natal habitat and have been observed in temporary ponds following longer dry periods (Crumpton 1979, Rowe 1987, Chapters Two and Three). These flexible development and anti-predator traits combined with an ability to tolerate drying indicate that *X. zealandica* has juvenile adaptations to exploit a range of habitats across the pond-permanence gradient. Juveniles have broad pond distributions, but there are also constraints to their developmental flexibility due to drying disturbances and predator avoidance behaviours. Larvae can be extirpated from ponds that experience longer dry periods and despite this risk adults readily oviposit in unpredictable temporary ponds, resulting in population turnover. Studies of amphibians and invertebrates have found small freshwater habitats with low connectivity had reduced allelic richness and newly colonised populations had greater genetic divergence

resulting from founder effects associated with turnover (Shama et al. 2011, Cosentino et al. 2012). *Xanthocnemis zealandica* may experience population turnover in unpredictable temporary ponds, but hydroperiod did not influence their allelic richness or population structure, suggesting they have high connectivity between habitat types. Widespread dispersal between habitat types combined with *X. zealandica*'s flexible development and anti-predator behaviours, and short-term desiccation tolerance maybe overriding local environmental influences and may result in overall weak selection pressures, thereby reducing population divergence across the pond-permanence gradient.

Potential gene flow/recent bottlenecks

Variable habitat predictability can lead to uneven gene flow among habitats with different abiotic and biotic selection pressures with temporary habitats more prone to population turnover and founder effects with dispersal from more permanent habitat sources resulting in potential population bottlenecks (Shama et al. 2011). However, dispersal capability between habitats may be more important than environmental tolerance to maintain distributions across a range of habitats and counter potential population bottlenecks (Huey et al. 2011, Arribas et al. 2012b).

Xanthocnemis zealandica had evidence of a strong dispersal capability with both long-distance and local gene flow between habitat types that reinforced the population structure results. At the regional scale, there was no contemporary gene flow among the islands, but within the South Island, Cass was the main source of migrants to both neighbouring pond complexes. This indicates that *X. zealandica* can undergo long-distance dispersal among pond complexes.

Studies of aquatic invertebrate dispersal distances and habitat selection have found they can favour longer dispersal distances rather than colonising immediate neighbouring habitats possibly due to the potential benefits of colonising more distant habitats that might not have similar selection pressures (McCauley et al. 2009). Additionally, uneven spatial distribution of populations experiencing different extinction regimes (e.g., permanent and temporary ponds) should favour long-distance dispersers that might have a greater chance of settling in habitats with more favourable environmental conditions (Rousset and Gandon 2002). However, if long distance dispersers reach unsuitable sites there will be an extra cost to long-distance dispersal and will likely select for a lower average dispersal distance (Hoverstadt et al. 2001, Rousset and Gandon 2002). While *X. zealandica* may benefit from dispersal among habitat types to counter local ecological influences their gene flow was not homogeneous.

Within the pond complexes, *X. zealandica* typically had greater migration from permanent ponds, though Cass was an exception with more gene flow from a temporary pond. Permanent ponds maybe greater sources of migrants due to their larger size and potentially contribute a greater number of individuals to the regional gene pool. Interestingly, exchange of migrants at both spatial scales was consistently uni-directional and not all permanent ponds were sources of migrants. There was also little evidence of gene flow to the outlier populations (Hakatere: Lambies 1, Tarndale: Bowscale Tarn). This lack of gene flow with these populations may explain the higher levels of inbreeding and unique population structure; however, there were no obvious geographic barriers that should have inhibited gene flow with these ponds. This suggests that there may be other environmental factors such as local climate patterns (e.g., wind direction) or adult dispersal behaviours that have caused asymmetrical gene flow among ponds. For example, uneven gene flow could have resulted from varying natal habitat conditions, such as a higher propensity of dispersal away from ponds containing predatory fish, higher mortality of immigrants to permanent ponds due to

fish, or differences in territorial behaviours between habitats (McPeek 1989, Benard and McCauley 2008, McCauley and Rowe 2010). Alternatively, these gene flow estimates may be under-estimating contemporary gene flow. Faubet et al. (2007) warns that BAYESASS models have limited power to provide meaningful migration estimates with real-world scenarios, especially those that involve low levels of genetic differentiation, therefore these estimates should be interpreted with caution. Given that *X. zealandica* adults were commonly observed ovipositing in temporary ponds that experienced extirpations suggests that the general pattern of high gene flow between habitat types may be real (Crumpton 1979, Rowe 1987, Chapter Two).

These results complement similar studies of freshwater organisms that had low population structure due to their high gene flow across a range of habitats (Seppa and Laurila 1999, De Block et al. 2005, Huey et al. 2011). For example, two species of dryland fish (*Maquaria ambigua* and *Tandanus tandanus*) and a crustacean (*Macrobrachium australiense*) had weak genetic structure as a result of high gene flow among waterholes, despite experiencing population turnover from river drying (Huey et al. 2011). Furthermore, another damselfly (*Calopteryx splendens*) had similarily high migration rates (> 0.2) and this high gene flow likely counteracted local genetic differentiation (Svensson et al. 2014). This contrasts to dispersal limited freshwater organisms that experience low connectivity across multiple habitats, resulting in greater population differentiation (Zickovich and Bohonak 2007, Cosentino et al. 2012). Freshwater organisms that have limited population differentiation due to their strong dispersal abilities and bet-hedging behaviours also may be less likely to have specialised adaptations specific to temporary or permanent habitat conditions (De Block et al. 2005). De Block et al. (2005) attributed the lack of local adaptation of a damselfly (*Lestes viridis*) to temporary pond conditions to high gene flow between populations experiencing metapopulation source-sink dynamics in permanent and temporary ponds. While low or even

moderate levels of gene flow can result in adaptation and genetic divergence to distinct habitats, high levels of dispersal between habitats can swamp contemporary adaptation, reduce genetic drift, and reduce specialisation (Garant et al. 2007, Blanquart et al. 2012). Furthermore, the evolution of niche-breadth and dispersal rates are expected to be positively related (Holt and Gomulkiewicz 1997, Holt et al. 2004). These models have been supported by empirical evidence of higher dispersal propensity by generalist species (McCauley 2007, Entling et al. 2011). High dispersal rates across heterogeneous habitats may also reduce the susceptibility of generalist species to potential population bottlenecks from unpredictable disturbances.

The extent of connectivity between habitats can influence whether unpredictable disturbances can lead to population bottlenecks resulting from turnover and subsequent founder effects. Population bottlenecks might be more common in habitats that experience frequent unpredictable disturbances, but if there is extensive gene flow between multiple habitats there may be little evidence of founder effects (Harrison and Hastings 1996, Huey et al. 2011, Blaquart et al. 2013). There was no evidence of *X. zealandica* short-term bottlenecks in either the pond complexes or individual ponds, even though temporary ponds in this study have historically experienced extirpation. During drier years these habitats do not retain sufficient water to sustain complete *X. zealandica* juvenile development according to records from aquatic community sampling and subsequent population monitoring (Greig 2008, Wissinger et al. 2009, Chapter Two). This inter-annual variability suggests temporary pond populations can exist in boom-bust cycles due to unpredictable hydrology and subsequent recolonisation from neighbouring pond sources. This pattern of meta-population connectivity is a common strategy for species persisting in extirpation-prone environments and has been found in other temporary pond and intermittent stream invertebrate populations (Harrison and Hastings 1996, De Block et al. 2005, Huey et al. 2011). Therefore, *X. zealandica* may have low

bottlenecking risk, or the extent pond drying is not severe enough to influence effective meta-population sizes. This contrasts to other aquatic invertebrates, such as Swiss alpine caddisflies (*Allogamus uncatus*) that experienced population bottlenecks from drought conditions (Shama et al. 2011). Severe droughts can result in both temporary and semi-permanent ponds drying, yet there may be sufficient connectivity with permanent ponds to rapidly recolonise and not cause population bottlenecks across the pond complexes. Thus, *X. zealandica* metapopulation dynamics may retain genetic diversity and limit their exposure to population bottlenecks despite frequent unpredictable disturbances.

For organisms that sustain populations across a variety of habitat types, dispersal may be more important than environmental influences in their natal habitats (Arribas et al. 2012b). Gene flow has been shown to sustain populations in unpredictable habitats that may experience inter-annual local extinctions followed by recolonisation from multiple habitat sources (Huey et al. 2011). My results suggest regional and local connectivity can retain more genetic diversity and limit population differentiation more than might be expected in habitats that experience frequent stochastic events, thereby reducing the impacts of potential bottlenecks. Therefore, widespread adult dispersal could be critical for generalist species that exploit a range of environments, but the success of their offspring may depend on juvenile life-history traits to adapt to, or counter, contrasting selection pressures in their natal habitat (Sultan and Spencer 2002, De Block et al. 2005, Benard and McCauley 2007). In previous studies, I found *X. zealandica* had flexible life-history traits (i.e. flexible development and anti-predator behaviour) to respond to drying and predator stressors (Chapters Two and Three). This combination of strong adult dispersal and flexible life-history traits could be mechanisms whereby generalist invertebrates minimise local selection pressures, allowing them to persist across a wide range of environments and dominate New Zealand temporary aquatic communities (Winterbourn et al. 1981, Wissinger et al. 2009). This study provides

insight into how generalist species can rely on gene flow among heterogenous freshwater environments to achieve broad distributions despite experiencing contrasting local selection pressures and resilience to unpredictable population turnover.

Appendix

Table 4.S1. Microsatellite loci characteristics including locus name, forward and reverse primer sequence, repeat motif, expected PCR product size, number indicating multiplex grouping, primer volumes used in multiplex PCR reactions, dye label using in multiplex, total number of alleles for each locus and their expected heterozygosity.

Locus	Primer sequence	Repeat motif	Expected PCR product size (bp)	Multiplex	[Primer] (uL)	Label	Alleles	H_{exp} (S.E)
XZ-10	F: TCCACCGTAGATTGCTGTCTC	$(AAT)_6$	191	1	0.006	VIC	13	0.523 (± 0.032)
	R: TTCAGCCACATCGTTAGCAG				0.024			
XZ-14	F: AGCTCGGGAGAGAACACG	$(AG)_9$	158	1	0.008	FAM	14	0.739 (± 0.024)
	R: AAATACGATCGCGGATGC				0.032			
XZ-17	F: GCGCTTAGCCACCTCTGA	$(AG)_9$	217	1	0.008	FAM	18	0.739 (± 0.006)
	R: CCCGGCAAGCGTTATTTC				0.032			
XZ-19	F: AGAGGCCTTCTTCCTTCCAG	$(AG)_9$	226	1	0.008	NED	10	0.704 (± 0.026)
	R: CCACCTCCTGCTTCGTAGTC				0.032			
XZ-23	F: CCAGCCATTGGTCCAACTAT	$(AG)_9$	240	1	0.006	VIC	10	0.690 (± 0.012)
	R: TGACAGTGACGTTGGAAACC				0.024			
XZ-04	F: CTACGCAGTTACCCTCCAATTC	$(ATC)_6$	224	2	0.006	VIC	24	0.690 (± 0.026)
	R: ACGGTAAAGGGAGACCTCGTAT				0.024			
XZ-11	F: GAGCAGAGTGCAGCTGAATG	$(ATC)_6$	233	2	0.008	NED	20	0.367 (± 0.029)
	R: TCGTATGCGATACGTGGAAC				0.032			
XZ-03	F: TCTCCGACCTAGTACTGACAAGTG	$(AAT)_7$	217	3	0.008	FAM	13	0.459 (± 0.038)
	R: GACCAACTGACCACGTACTTACAG				0.032			
Mean							15.25	0.621 (± 0.014)

Table 4.S2. Chi-square tests of deviation from Hardy Weinberg equilibrium (HWE) in microsatellite loci of *Xanthocnemis zealandica*. Significant deviations after adjusted for multiple tests by a Dunn-Sidak correction (n = 176): $p = 0.000291$, indicated in bold and an asterisks.

	XZ10	XZ14	XZ17	XZ19	XZ23	XZ11	XZ04	XZ03
MK04	4.01	3.48	8.40	17.78	11.06	17.27	**47.12***	0.02
MK05	5.82	**29.67***	23.32	**32.44***	10.70	30.37	11.68	0.59
MK01	13.32	17.71	20.85	**52.23***	7.25	11.42	7.73	0.11
MK03	7.23	5.06	19.83	**32.07***	13.91	9.72	22.77	0.25
TB1	3.56	18.36	18.76	32.89	1.68	**16.00***	42.00	0.12
TS	**28.96***	**72.55***	24.05	**70.95***	8.29	34.26	**60.46***	5.64
TB4	24.46	24.36	56.52	50.11	15.64	**21.26***	52.62	1.18
TBT	16.77	23.09	11.45	26.66	5.29	5.84	4.11	**20.30***
TR	0.44	33.95	22.59	39.95	6.92	21.58	17.63	0.14
T3T2	1.51	10.76	30.09	24.96	3.70	29.82	**91.41***	5.85
CS	21.01	17.93	44.34	29.09	12.09	7.99	**81.87***	16.24
CH	9.42	23.49	43.09	**48.67***	5.63	4.65	0.96	12.94
CHR	4.50	54.60	59.22	33.97	9.30	14.03	39.78	1.87
CGO	10.51	33.00	68.25	30.46	12.39	0.32	30.10	2.27
CHM	29.02	23.98	33.14	41.78	12.25	**46.05***	55.67	2.36
CK	11.34	20.55	20.59	34.58	29.71	1.14	**50.91***	2.22
HR	**46.65***	12.93	23.65	**65.39***	4.89	**22.08***	**39.74***	4.95
HD	31.19	29.08	26.04	**76.91***	12.27	22.66	**71.47***	3.03
HFD	33.82	32.77	34.61	44.23	18.53	7.55	59.55	0.99
HTS	25.87	20.55	16.64	10.86	4.69	11.23	40.09	8.77
HDT	1.25	23.34	18.41	**52.08***	12.96	**18.00***	**39.67***	3.80
HL1	28.95	14.17	11.88	8.03	12.54	13.13	**13.00***	2.78

Table 4.S3. Pairwise G_{ST} (below diagonal) and Jost's *D* (above diagonal) matrix organised from the most northern pond complex (Makirikiri) to the most southern (Hakatere) and by pond hydroperiod (P = Permanent, S = Semi-permanent, T = Temporary). Significant pairwise combinations after Dunn-Sidak corrections for multiple tests indicated in bold.

Complex			Makirikiri				Tarndale						Cass						Hakatere					
	Habitat		P	P	T	T	P	P	S	S	T	T	P	P	S	S	T	T	P	P	S	S	T	T
		Pond	MK04	MK05	MK01	MK03	TB1	TS	TB4	TBT	TR	T3T2	CS	CH	CHR	CGO	CHM	CK	HR	HD	HFD	HTS	HDT	HL1
Maki	P	MK04	-	-0.006	**0.039**	-0.009	**0.267**	**0.177**	**0.213**	**0.313**	**0.269**	**0.213**	**0.198**	0.202	**0.219**	**0.200**	**0.232**	**0.291**	0.213	**0.208**	**0.212**	**0.243**	**0.215**	**0.286**
	P	MK05	0.001	-	**0.059**	-0.008	**0.245**	**0.166**	**0.213**	**0.292**	**0.258**	**0.217**	**0.207**	**0.185**	**0.227**	**0.226**	**0.220**	**0.302**	**0.221**	**0.193**	**0.214**	**0.265**	**0.226**	**0.294**
	T	MK01	0.010	**0.018**	-	0.014	**0.341**	**0.259**	**0.285**	**0.368**	**0.342**	**0.315**	**0.260**	**0.278**	**0.260**	**0.286**	**0.270**	**0.321**	**0.265**	**0.250**	**0.278**	**0.243**	**0.313**	**0.341**
	T	MK03	-0.003	0.001	0.003	-	**0.292**	**0.202**	**0.222**	**0.326**	**0.311**	**0.258**	**0.218**	**0.208**	**0.236**	**0.243**	**0.221**	**0.314**	0.242	**0.223**	**0.227**	**0.261**	**0.243**	**0.303**
Tarn	P	TB1	**0.114**	**0.092**	**0.135**	**0.111**	-	0.011	0.028	**0.138**	0.060	0.052	0.028	**0.108**	**0.102**	0.046	**0.083**	**0.076**	**0.100**	0.062	0.040	0.131	0.061	**0.238**
	P	TS	**0.062**	**0.052**	**0.087**	**0.064**	0.010	-	0.009	**0.178**	**0.053**	0.021	0.019	0.029	**0.050**	**0.044**	**0.046**	**0.084**	**0.045**	**0.052**	**0.032**	**0.079**	0.029	**0.226**
	S	TB4	**0.079**	**0.066**	**0.103**	**0.074**	0.000	0.005	-	**0.197**	**0.113**	**0.057**	0.020	**0.067**	0.035	0.036	0.037	**0.093**	**0.098**	**0.108**	**0.092**	**0.106**	**0.080**	**0.184**
	S	TBT	**0.101**	**0.088**	**0.119**	**0.099**	**0.032**	**0.039**	**0.041**	-	**0.192**	**0.175**	**0.184**	**0.220**	**0.216**	**0.202**	**0.200**	**0.221**	**0.167**	**0.166**	**0.169**	**0.221**	**0.193**	**0.122**
	T	TR	**0.119**	**0.104**	**0.139**	**0.119**	0.012	**0.017**	**0.027**	**0.063**	-	**0.040**	**0.065**	**0.085**	**0.072**	**0.052**	**0.086**	**0.053**	**0.040**	**0.102**	**0.098**	**0.064**	0.032	**0.278**
	T	T3T2	**0.081**	**0.072**	**0.113**	**0.088**	0.005	0.004	0.008	**0.043**	**0.010**	-	**0.063**	**0.098**	**0.045**	**0.037**	**0.052**	**0.070**	**0.045**	**0.066**	**0.054**	**0.063**	0.034	**0.228**
Cass	P	CS	**0.070**	**0.061**	**0.087**	**0.066**	0.006	**0.011**	0.002	**0.045**	**0.022**	**0.015**	-	0.014	0.027	0.013	0.030	**0.068**	**0.044**	**0.037**	0.019	**0.062**	0.017	**0.204**
	P	CH	0.057	**0.049**	**0.080**	**0.056**	**0.040**	0.011	**0.019**	**0.066**	**0.039**	**0.026**	0.011	-	**0.056**	**0.104**	0.051	**0.148**	0.043	0.047	0.047	**0.117**	**0.062**	**0.248**
	S	CHR	**0.079**	**0.069**	**0.095**	**0.075**	**0.017**	**0.012**	0.005	**0.054**	**0.015**	**0.009**	0.002	**0.013**	-	0.007	-0.018	0.026	0.019	**0.058**	0.039	0.014	0.030	**0.184**
	S	CGO	**0.098**	**0.086**	**0.122**	**0.098**	-0.003	**0.017**	0.004	**0.047**	**0.013**	**0.007**	0.006	**0.035**	0.004	-	0.030	0.007	**0.037**	**0.054**	**0.037**	0.037	0.000	**0.205**
	T	CHM	**0.084**	**0.069**	**0.102**	**0.077**	**0.013**	**0.015**	0.005	**0.049**	**0.025**	**0.013**	0.006	0.014	-0.002	0.006	-	**0.040**	**0.051**	**0.048**	**0.041**	**0.057**	0.042	**0.195**
	T	CK	**0.132**	**0.116**	**0.141**	**0.126**	0.006	**0.034**	**0.021**	**0.060**	**0.016**	**0.023**	**0.019**	**0.051**	**0.012**	0.002	**0.013**	-	**0.040**	**0.067**	**0.076**	0.029	0.036	**0.230**
Haka	P	HR	**0.079**	**0.071**	**0.094**	**0.079**	**0.030**	**0.012**	**0.024**	**0.057**	**0.013**	**0.013**	**0.013**	**0.014**	0.003	**0.017**	**0.016**	**0.022**	-	0.018	**0.034**	0.035	0.008	**0.237**
	P	HD	**0.062**	**0.050**	**0.076**	**0.060**	**0.020**	**0.017**	**0.020**	**0.042**	**0.036**	**0.022**	**0.009**	0.011	0.011	**0.019**	**0.009**	**0.028**	0.010	-	0.014	**0.066**	**0.064**	**0.245**
	S	HFD	**0.071**	**0.060**	**0.086**	**0.066**	**0.011**	**0.008**	**0.013**	**0.039**	**0.024**	**0.014**	0.002	0.012	0.003	**0.012**	**0.009**	**0.025**	0.006	0.007	-	**0.061**	0.013	**0.237**
	S	HTS	**0.081**	**0.079**	**0.076**	**0.075**	**0.035**	**0.031**	**0.030**	**0.062**	**0.022**	**0.028**	0.016	**0.037**	0.010	**0.024**	**0.029**	**0.024**	0.012	**0.025**	**0.016**	-	0.051	**0.195**
	T	HDT	**0.086**	**0.075**	**0.109**	**0.086**	0.008	0.007	**0.011**	**0.049**	0.007	0.005	0.005	0.020	0.002	0.001	0.009	**0.012**	0.002	**0.019**	0.002	0.016	-	**0.227**
	T	HL1	**0.103**	**0.093**	**0.118**	**0.100**	**0.063**	**0.058**	**0.048**	**0.029**	**0.092**	**0.062**	**0.047**	**0.071**	**0.053**	**0.060**	**0.052**	**0.077**	**0.074**	**0.057**	**0.053**	**0.057**	**0.060**	-

Table 4.S4. Isolation-by-distance at the three hierarchical spatial scales (South Island and individual pond complexes) for Nei's genetic distance vs. Euclidean geographical distances (kilometers and log-transformed kilometers).

Pond complex	# Sites	Distance (km)		Distance (LOG(km))	
		r_{xy}	*p* - value	r_{xy}	*p* - value
South Island	18	0.140	0.054	0.006	0.386
Makirikiri	4	0.745	0.084	0.744	0.084
Tarndale	6	-0.034	0.425	-0.017	0.438
Cass	6	-0.132	0.327	-0.215	0.151
Hakatere	6	-0.453	0.130	-0.446	0.143

Table 4.S5. Proportion of emigrants and immigrant estimates among pond complexes (A) and within each pond complex (B-E) were based on unidirectional assignments of microsatellite genotypes in BAYESASS v.3.0.3, with the source population above and recipient population below. The proportion of non-migrants is represented in grey. Significant estimates of migrants and their 95% CI values are in bold. The habitat type, (P: permanent, S: semi-permanent, and T: temporary) are indicated for each pond.

A) Pond complex

	Makirikiri	**Tarndale**	**Cass**	**Hakatere**
Makirikiri	0.981	0.004	0.010	0.004
	(0.9621 - 0.9977)	(4.48e-8 - 0.0133)	(5.29e-7 - 0.0257)	(6.89e-8 - 0.0128)
Tarndale	0.006	0.824	**0.165**	0.005
	(5.59e-7 - 0.0175)	(0.7643 - 0.8851)	**(0.1035 - 0.2264)**	(1.46e-7 - 0.0142)
Cass	0.007	0.013	0.969	0.011
	(5.67e-7 - 0.0184)	(7.88e-6 - 0.031)	(0.9415 - 0.9925	(2.11e-8 - 0.0314)
Hakatere	0.005	**0.091**	**0.226**	0.678
	(3.95e-7 - 0.0146)	**(0.0446 - 0.1419)**	**(0.1759 - 0.2729)**	(0.6667 - 0.7011)

B) Makirikiri

Pond		**MK04**	**MK05**	**MK01**	**MK03**
	Habitat	P	P	T	T
MK04	P	0.685	**0.273**	0.023	0.020
		(0.667 - 0.718)	**(0.206 - 0.328)**	(4.80e-8 - 0.068)	(1.39e-6 - 0.057)
MK05	P	0.019	0.921	0.022	0.039
		(7.46e-7 - 0.051)	(0.846 - 0.991)	(5.49e-8 - 0.062)	(4.08e-7 - 0.096)
MK01	T	0.019	**0.236**	0.714	0.031
		(1.02e-6 - 0.056)	**(0.152 - 0.315)**	(0.667 - 0.784)	(1.15e-7 - 0.082)
MK03	T	0.019	**0.271**	0.024	0.686
		(1.01e-7 - 0.055)	**(0.209 - 0.326)**	(6.52e-7 - 0.067)	(0.667 - 0.723)

C) Tarndale

Pond		TB1	TS	TB4	TBT	TR	T3T2
	Habitat	P	P	S	S	T	T
TB1	P	0.645	**0.212**	0.023	0.023	0.022	0.028
		(0.667 - 0.741)	**(0.128 - 0.293)**	(4.56e-7 - 0.065)	(3.39e-9 - 0.067)	(8.59e-7 - 0.062)	(1.24e-6 - 0.077)
TS	P	0.011	0.930	0.011	0.017	0.012	0.019
		(5.62e-7 - 0.033)	(0.878 - 0.981)	(3.22e-8 - 0.032)	(6.71e-7 - 0.047)	(4.67e-7 - 0.034)	(1.12e-6 - 0.055)
TB4	S	0.012	**0.262**	0.680	0.019	0.012	0.016
		(1.44e-7 - 0.034)	**(0.206 - 0.309)**	(0.667 - 0.705)	(2.76e-6 - 0.050)	(6.05e-7 - 0.035)	(2.15e-7 - 0.047)
TBT	S	0.017	0.020	0.018	0.902	0.018	0.026
		(1.79e-7 - 0.050)	(6.94e-7 - 0.056)	(1.58e-6 - 0.051)	(0.835 - 0.963)	(4.07e-7 - 0.053)	(4.28e-7 - 0.068)
TR	T	0.012	**0.264**	0.012	0.016	0.680	0.016
		(5.83e-7 - 0.035)	**(0.209 - 0.313)**	(2.29e-8 - 0.036)	(7.02e-9 - 0.045)	(0.667 - 0.706)	(2.42e-8 - 0.046)
T3T2	T	0.009	**0.282**	0.009	0.013	0.009	0.679
		(9.33e-8 - 0.027)	**(0.238 - 0.319)**	(7.30e-8 - 0.027)	(1.56e-6 - 0.036)	(7.13e-7 - 0.026)	(0.667 - 0.703)

D) Cass

Pond		CS	CH	CHR	CGO	CHM	CK
	Habitat	P	P	S	S	T	T
CS	P	0.702	0.010	0.010	0.011	0.034	**0.234**
		(0.667 - 0.763)	(1.22e-7 - 0.029)	(5.09e-7 - 0.029)	(4.19e-7 - 0.032)	(3.20e-7 - 0.092)	**(0.170 - 0.295)**
CH	P	0.104	0.688	0.018	0.018	0.072	0.101
		(6.87e-7 - 0.246)	(0.667 - 0.727)	(5.87e-7 - 0.053)	(3.46e-7 - 0.053)	(5.52e-7 - 0.224)	(0.001 - 0.218)
CHR	S	0.025	0.013	0.681	0.013	0.022	**0.245**
		(4.57e-6 - 0.077)	(2.10e-7 - 0.039)	(0.667 - 0.707)	(4.77e-8 - 0.039)	(1.69e-6 - 0.067)	**(0.175 - 0.307)**
CGO	S	0.012	0.010	0.011	0.678	0.013	**0.276**
		(8.12e-7 - 0.036)	(4.29e-7 - 0.031)	(3.28e-7 - 0.031)	(0.667 - 0.700)	(1.26e-9 - 0.038)	**(0.229 - 0.317)**
CHM	T	0.023	0.010	0.011	0.011	0.688	**0.257**
		(2.18e-6 - 0.069)	(4.18e-9 - 0.030)	(2.75e-7 - 0.032)	(6.63e-7 - 0.033)	(0.667 - 0.726)	**(0.197 - 0.311)**
CK	T	0.020	0.011	0.012	0.014	0.019	0.923
		(3.17e-7 - 0.057)	(4.43e-7 - 0.034)	(3.29e-7 - 0.035)	(7.81e-7 - 0.042)	(3.45e-7 - 0.056)	(0.866 - 0.978)

E) Hakatere

Pond		HR	HD	HFD	HTS	HDT	HL1
	Habitat	P	P	S	S	T	T
HR	P	0.920	0.025	0.018	0.012	0.013	0.012
		(0.857 - 0.976)	(4.77e-7 - 0.069)	(4.54e-7 - 0.052)	(7.58e-8 - 0.036)	(5.37e-7 - 0.037)	(1.56e-6 - 0.036)
HD	P	**0.233**	0.700	0.026	0.012	0.014	0.015
		(0.162 - 0.296)	(0.667 - 0.755)	(7.07e-8 - 0.071)	(1.26e-6 - 0.036)	(2.90e-7 - 0.039)	(2.13e-6 - 0.043)
HFD	S	**0.273**	0.016	0.681	0.010	0.010	0.010
		(0.222 - 0.316)	(1.25e-8 - 0.047)	(0.667 - 0.708)	(3.28e-7 - 0.030)	(3.20e-7 - 0.029)	(1.26e-7 - 0.030)
HTS	S	**0.186**	0.027	0.038	0.688	0.018	0.043
		(0.094 - 0.269)	(4.12e-7 - 0.074)	(1.27e-5 - 0.089)	(0.667 - 0.728)	(8.58e-8 - 0.054)	(8.31e-4 - 0.094)
HDT	T	**0.233**	0.018	0.037	0.015	0.684	0.015
		(0.167 - 0.294)	(2.29e-7 - 0.052)	(8.66e-6 - 0.082)	(1.29e-7 - 0.042)	(0.667 - 0.716)	(1.37e-8 - 0.044)
HL1	T	0.018	0.030	0.020	0.019	0.018	0.895
		(5.59e-7 - 0.053)	(1.28e-6 - 0.082)	(5.11e-7 - 0.057)	(5.45e-7 - 0.055)	(4.27e-8 - 0.053)	(0.819 - 0.967)

Table 4.S6. The probability that loci fit the stepwise mutation (SMM), two-phase (TPM) models of mutation-drift equilibrium and the mode-shift test conducted with BOTTLENECK v.1.2.02 for all *Xanthocnemis zealandica* loci analyzed for each pond population (A) and grouped at the pond complex level (B). Deviations from these assumptions indicate a possible recent bottleneck. Wilcoxon's tests were evaluated for one-tailed probabilities for heterozygosity excess.

	Wilcoxon's Test		
	SMM	TPM	Mode-shift test
A) Pond			
MK04	0.996	0.980	L-shaped
MK05	0.992	0.980	L-shaped
MK01	0.813	0.594	L-shaped
MK03	0.973	0.973	L-shaped
T3T2	0.996	0.988	L-shaped
TB1	0.766	0.711	Shifted mode
TB4	0.992	0.988	L-shaped
TBT	0.973	0.973	L-shaped
TR	0.973	0.852	L-shaped
TS	0.961	0.852	L-shaped
CGo	0.996	0.992	L-shaped
CH	0.980	0.852	L-shaped
CHM	0.961	0.852	L-shaped
CHR	0.988	0.973	L-shaped
CK	0.996	0.992	L-shaped
CS	0.961	0.813	L-shaped
HD	0.988	0.980	L-shaped
HDT	0.988	0.988	L-shaped
HFD	0.988	0.973	L-shaped
HL1	0.961	0.945	L-shaped
HR	0.945	0.813	L-shaped
HTS	0.980	0.980	L-shaped
B) Complex			
Makirikiri	0.996	0.992	L-shaped
Tarndale	1.000	1.000	L-shaped
Cass	1.000	1.000	L-shaped
Hakatere	1.000	0.988	L-shaped

Plate 8. Adult *Xanthocnemis zealandica* hiding behind a leaf of grass in a Hakatere pond.

Chapter Five:

Unpredictable disturbances ameliorate life-history trade-offs, increase niche-width, influence speciation, and affect responses to climate warming

Habitat-permanence gradients, such as those associated with drying disturbances in ponds, can result in distinct communities with species adapted to either temporary or permanent habitats depending on how they solve associated trade-offs, but there are some organisms, generalists, that can persist across a wide range of environments (Figure 5.1). The mechanisms that permit these generalists to sustain broad realised niches across habitats with contrasting local selection pressures have been poorly understood, but may depend on the predictability of environmental disturbances (Kassen 2002, Debarre and Gandon 2011). To sustain populations across unpredictable disturbance gradients, generalist species may rely on a combination of juvenile life-history strategies and adult dispersal. Moreover, how juvenile life-history strategies and adult dispersal allow generalist species to counter contrasting selection pressures associated with disturbance provides insights on how unpredictable disturbances under global changes might influence population persistence, species evolution, and community ecology. Here I outline the evidence for these conclusions based on my studies of generalist invertebrate life-history trade-offs across a gradient of pond-drying disturbance (Chapters Two to Four).

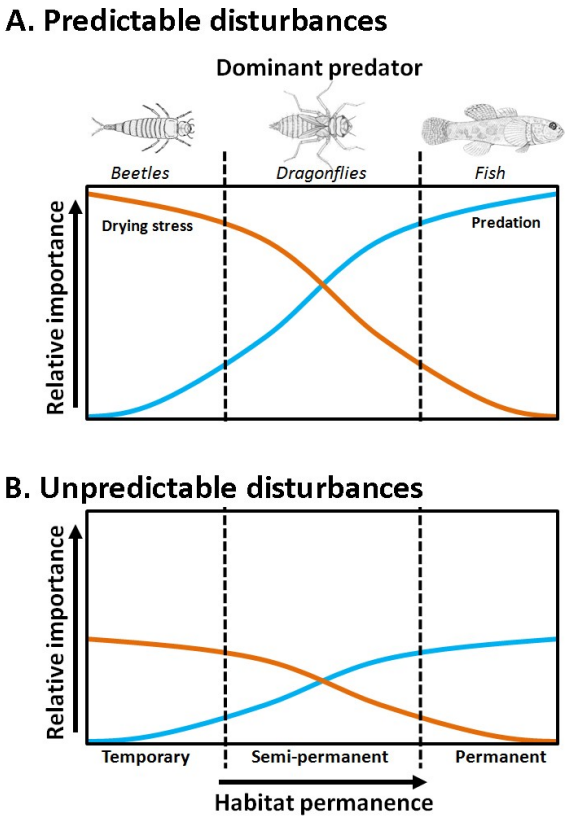

Figure 5.1 Relative importance of contrasting abiotic (drying stress, orange line) and biotic (dominant predators, blue line) selection pressures influencing invertebrate life-history trade-offs across a habitat-permanence gradient in systems with (A) predictable and (B) unpredictable disturbances. Systems experiencing predictable disturbances typically follow the classic Wellborn et al. (1996) model where contrasting environmental stressors result in species replacements along the habitat-permanence gradient, where specialists trade-off drying tolerance for predator avoidance or vice-versa. Diverging from the original model, unpredictable systems may be dominated by generalist invertebrates which can moderate local selection pressures through adult dispersal and alternative juvenile life-history traits. These traits can include flexible development and anti-predator behaviours, or inflexible rapid development and ready dispersal, to exploit the range of habitats across the habitat-permanence gradient.

These studies revealed aquatic invertebrates can use alternative juvenile life-history traits and widespread adult dispersal to counterbalance local selection pressures across an unpredictable pond disturbance gradient. However, the developmental- and behavioural-flexibility of two pond invertebrates (*Xanthocnemis zealandica* damselflies and *Sigara arguta* waterboatmen) to contrasting drying and predator stressors associated with the disturbance gradient depended on their phenology, which influenced their distributions along the disturbance gradients associated with pond-permanence (Chapter Two and Three). *Xanthocnemis zealandica*'s flexible life-history strategy with plastic juvenile development periods, variable anti-predator behaviours, and short-term desiccation tolerance, likely resulted from their longer exposure to selection pressures in their natal habitats during their nymph stages. Yet, there were limits to *X. zealandica*'s flexibility and they were unable to complete development in some temporary ponds with inadequate growth days (< 135 days) due to long periods of habitat drying disturbance (Chapter Two; Figure 5.2C). In contrast, *Sigara arguta* had an inflexible strategy and experienced high mortality due to predatory fish and desiccation regardless of natal habitat. Nevertheless, *S. arguta*, through their rapid development and early colonisation, were able to occupy temporary ponds, and by using shallow sub-habitats were also able to live in permanent ponds (Figure 5.2D). Thus, although these two invertebrates had reasonably different life-history strategies, both species were able to exploit a relatively wide variety of ponds ranging from temporary ponds with unpredictable inter- and intra-annual drying to permanent ponds containing predatory fish. Through amelioration of the life-history trade-offs traditionally associated with predictable disturbances these species likely experienced weak selection pressures to specialise traits specific to either temporary or permanent pond conditions (Figure 5.1B). This situation likely favours generalist traits when species are confronted with unpredictable environmental disturbances (de Meeus and Goudet 2000, Debarre and Gandon 2011, Massol 2013).

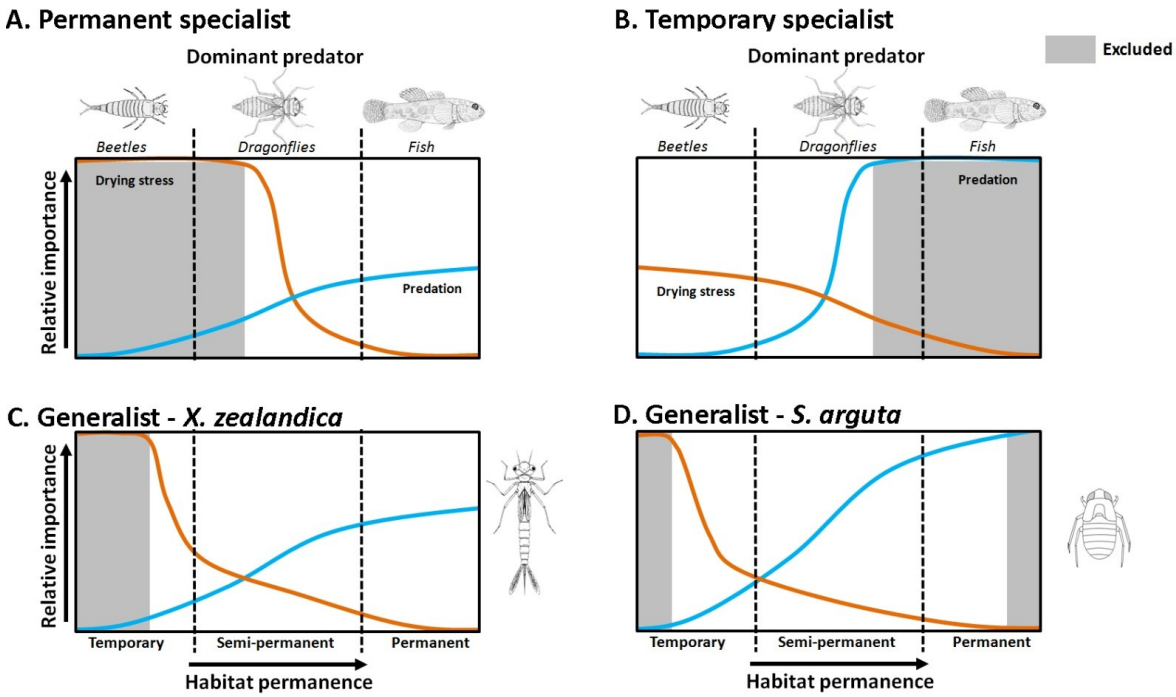

Figure 5.2. Distribution of pond invertebrates with specialist life-history traits (A-B) according to a modified Wellborn et al. (1996) predator-permanence gradient, compared to two New Zealand species with generalist life-history traits, *X. zealandica* (C) and *S. arguta* (D), where contrasting drying stress (orange line) and predation (blue line) can result in habitat exclusions (grey). In the classic predator-permanence gradient model, species replacements result from specialists that trade-off drying tolerance (e.g., desiccation tolerance, rapid development; A) or predator avoidance (e.g., anti-predator morphology, behaviours, etc.; B). In contrast, I found two generalist species lack trade-offs due to particular life-history traits, allowing them to occupy the range of temporary, semi-permanent and permanent lentic habitats which characterise the pond predator-permanence gradient in New Zealand. The developmentally- and behaviourally-flexible *X. zealandica* were only excluded from temporary ponds with very short hydrologic regimes, but were able to coexist with predators in permanent ponds. *Sigara arguta* had an inflexible life-history strategy that allowed them to occupy ponds with shorter hydroperiods than *X. zealandica*. However, this also made *S. arguta* more susceptible to predatory fish, and they were excluded from some permanent ponds where *X. zealandica* were present.

In addition to alternative juvenile life-history strategies, both species I studied likely relied on recruitment between temporary and permanent habitats, with potential bet-hedging behaviours being used to maximise their offspring success among different habitats. For example, I found little evidence of *X. zealandica* genetic population structure or variable genetic diversity among different pond types, likely as a result of gene flow among and within pond complexes (Chapter Four). Furthermore, environmental predictors such as hydroperiod, fish presence, and pond size had little influence on population structure. This lack of genetic structure among freshwater habitats was not surprising for a strong disperser (Bohonak and Jenkins 2003, De Block et al. 2005, Davis et al. 2013). Overall, these types of metapopulation dynamics, with recruitment between neighbouring pond types, may also be critical to sustain regional populations of generalist invertebrates across an unpredictable gradient of disturbance.

Furthermore, although both species experienced extirpations from untimely or temporary pond drying, there was subsequent recolonisation from other pond sources (Chapter Two). In particular, permanent ponds likely acted as important sources of recruits, promoting gene flow (Chapter Four). However, during favourable (i.e. wet) years temporary pond populations could also be important contributors to the regional gene pool. This inter-annual variability in the hydrology of temporary ponds, which alters whether my study species experience a drying disturbance, could result in source-sink dynamics during drier years and reciprocal gene flow during wet years (Figure 5.3B). Therefore, unpredictable disturbances may generally favour adult bet-hedging behaviour, with adults dispersing and ovipositing between habitats to maximise fitness. Such behaviour was observed during the course of the field survey, with *X. zealandica* ovipositing in neighbouring permanent and dry temporary ponds (Plate 5). These results suggest that aquatic invertebrates can sustain metapopulations across an unpredictable disturbance gradient through dispersal among habitat types which might be

less likely in a more predictably disturbed system that presents strongly contrasting selection pressures (Figure 5.3A).

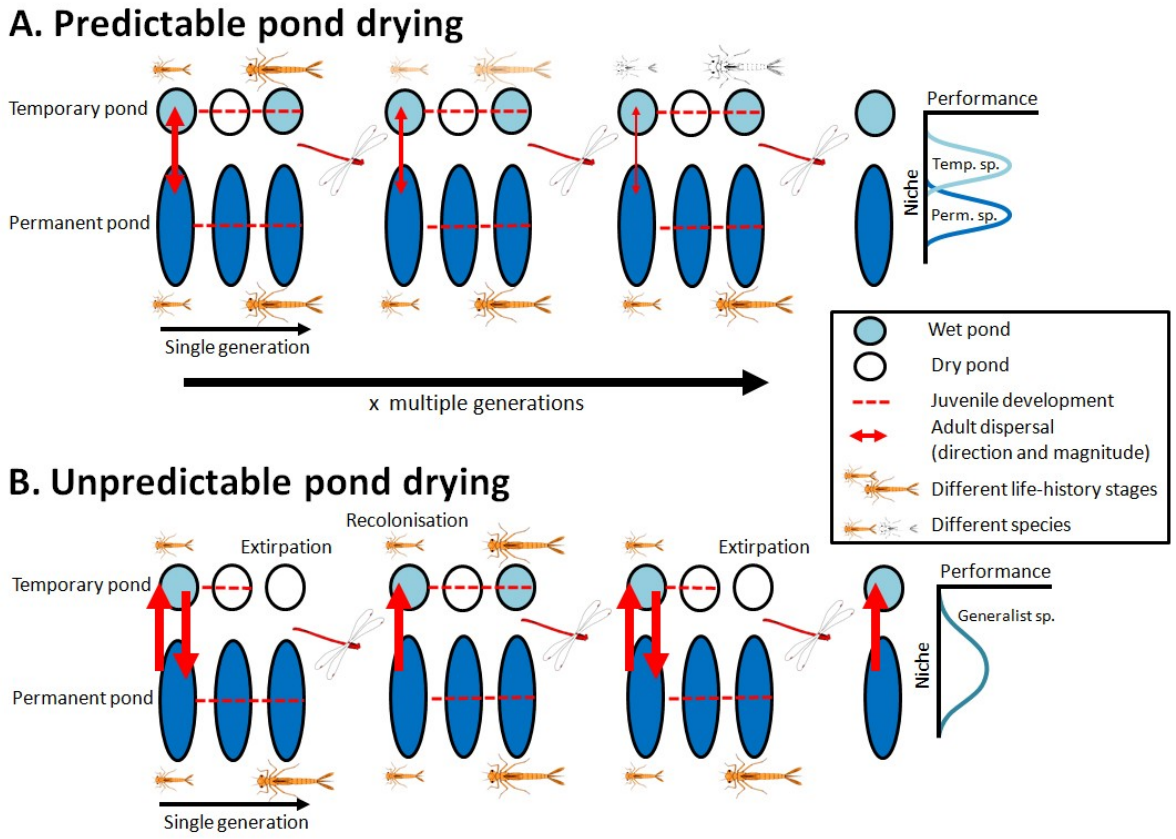

Figure 5.3. Potential mechanisms of speciation for an aquatic organism with a complex life-cycle linked to differences in habitat predictability across a gradient of hydrological habitat permanence. Under predictable disturbances (A), temporary habitats with reliable inter- and intra-annual drying disturbances over multiple generations should lead to less gene flow with permanent habitats and species divergence by habitat type. Populations experiencing unpredictable disturbances (B) on the other hand, might have populations across temporary and permanent habitats, sustained through source-sink metapopulation dynamics. Frequent unpredictable disturbances and recruitment among habitat types might limit habitat-specific adaptations and speciation. Dotted lines denote juvenile development over a generation.

The combination of juvenile life-history strategies that reduce local selection pressures, and gene flow across multiple habitat types through adult dispersal, are mechanisms which likely allow generalist species to persist across a range of environments experiencing unpredictable disturbances. Moreover, these same processes could limit local adaptation to local selection pressures and constrain speciation normally associated with habitat specialisation in more predictable systems (North et al. 2011, Thibert-Plante and Hendry 2011). Even moderate levels of gene flow can reduce population isolation and genetic drift, swamping adaptations to local conditions and causing maladapted phenotypes to be retained (Garant et al. 2007, Blanquart et al. 2012). Thus, although organisms with broad realised niches might be favoured under unpredictable environmental conditions, the frequency and severity of disturbances could also influence the retention of generalist traits.

Predictability of disturbances influences the role of generalist species

How pervasive generalist species are across a range of environments may also depend on the predictability of disturbances. According to paradigms of species distributions across predictable disturbance gradients, organisms should have adaptations specific to either disturbed (e.g., temporary ponds) or stable (e.g., permanent ponds) environmental conditions, resulting in species replacements between habitats experiencing different disturbance regimes (Peckarsky 1983, Menge and Sutherland 1987, Wellborn et al. 1996, Figure 5.1A). Yet, I found species can use generalist life-history strategies to exploit environments all along a gradient of disturbance, despite the contrasting selection pressures occurring along the gradient.

Species replacements typically arise in regions with predictable seasonal habitat disturbances such as temperate North American freshwater ecosystems with snowfall runoff or rainfall

occurring reliably at a particular time or season (Wissinger et al. 1999). When disturbances are predictable there is usually an advantage to adapt to local habitat selection pressures and less benefit for dispersal between habitat types (Figure 5.1A, 5.2A-B). In contrast, regions experiencing unpredictable intra- and inter-annual disturbances, such as New Zealand freshwater ecosystems, commonly have disturbed habitats dominated by species with generalist life-history traits that are a nested subset of neighbouring stable habitats (Winterbourn et al. 1981, Greig 2008, Wissinger et al. 2009, Arscott et al. 2010). With unreliable environmental cues and inter-annual population turnover and recruitment, these unpredictable conditions could limit local adaptations and reinforce gene flow between habitats. This suggests increasing the unpredictability of disturbances could result in a greater role for species with wider realised niches.

Different aspects of environmental disturbances (magnitude, frequency, predictability, rate of change, and duration) can also influence whether species evolve adaptations to avoid disturbances(Lytle and Poff 2004). Under predictable and frequent large magnitude disturbances species usually evolve synchronised life-history strategies to avoid disturbances. In contrast, disturbances that are frequent, high magnitude and unpredictable typically have low selection strength, especially in short-lived species that may not experience these conditions during their life-span (Lytle 2001, Lytle and Poff 2004). Nevertheless, environments that experience seasonal and superannual disturbances, such as intermittent desert streams and pools, can have species with specialised adaptations for long and severe disturbances or have transient life-history strategies to exploit narrow periods of favourable habitat conditions (Lake 2003, Bogan et al. 2011 and 2013, Robson et al. 2011, Davis et al. 2013). Thus, the distribution of generalist species across habitat-disturbance gradients depends on the predictability, frequency, and severity of environmental disturbances, but can

also be influenced by phylogeography and the strength of predation pressure in stable habitats, as has been found in New Zealand.

New Zealand context

Compared to freshwater invertebrate assemblages in other regions, New Zealand is remarkable for the prevalence of generalist taxa, which may be linked to selection pressures across disturbance gradients. Several explanations have been proposed for why New Zealand freshwater communities do not conform to the species replacement paradigm across habitat-permanence gradients, including phylogenetic constraints, highly unpredictable hydrology, and weak predator pressure in permanent habitats (Winterbourn et al. 1981, Wissinger et al. 2009, Arscott et al. 2010, Greig et al. 2013). In combination these conditions have likely resulted in ideal conditions for the persistence of generalist traits and may have ultimately limited specialisation along the habitat-permanence gradient, as I explain below.

Firstly, prevalence of freshwater invertebrates with generalist traits might be the result of phylogenetic constraints from New Zealand's geologic history and geographic isolation. Many of the current freshwater habitats in the South Island, New Zealand, were formed as recently as 15,000 years ago from the last major glacial retreat (Mosley 2004). Glacial processes, combined with New Zealand's geographic isolation, could explain the lack of diversity of aquatic fauna and limited species radiation (Rowe 1987, Wallis and Trewick 2009). This contrasts to other regions (e.g., North America) that had continental retreats from glacial advances providing more time for genetic divergence and speciation. In North America there is evidence of phylogenetic radiation of invertebrates with evolved specialised adaptations to temporary pond conditions from permanent-pond species with fish-avoidance (Brown et al. 2000, Turgeon et al. 2005).

Phylogenetic constraints might have limited the diversity of New Zealand fauna, but how species are able to persist across the habitat-permanence gradient still likely depends on life-history strategies that allow them to exploit a wide range of habitats with unpredictable hydrology and predatory fish. So secondly, frequent high magnitude unpredictable flooding and drying disturbances in New Zealand freshwaters provide unreliable environmental conditions which are likely to favour species with generalist life-history strategies. Hydrologic regimes of many of New Zealand's freshwaters are intrinsically linked to highly variable precipitation events which can result in unpredictable and high magnitude intra- and inter-annual flooding and habitat drying (Winterbourn et al. 1981, Wissinger et al. 2009, Arscott et al. 2010). These aseasonal disturbances result in unreliable environmental conditions that typically support species that can flexibly respond to shifting conditions or opportunistically exploit habitats during favourable periods, as I found in Chapters Two and Three.

Importantly, New Zealand freshwater habitats experience highly unpredictable wet periods, but not the extreme drying conditions like desert streams and rock pools that can have superannual drying with high temperatures and low humidity (Box et al. 2008, Bogan et al. 2011, Robson et al. 2011). These unpredictable and high magnitude or long duration disturbances can exert abiotic selection pressures beyond the constraints generalist life-history strategies can generally cope with, and so favour species with more specialised traits (Poff and Ward 1989, Lake 2003, Robson et al. 2011). Therefore, New Zealand seems to have a mix of frequent and unpredictable disturbances that are of sufficient duration and magnitude to result in weak abiotic selection pressures and limit more specialised traits, and thus leading to a dominance of generalists.

The third reason why generalist species might be so prevalent across habitat-permanence gradients may be linked to weak selection pressures in stable habitats. Being free from abiotic disturbances, stable environments typically have greater biotic selection pressures (Poff and Ward 1989, Wellborn et al. 1996). However, predators in New Zealand lakes and rivers (e.g., fish) may not impose strong selection on invertebrates. For example, native and introduced predatory fish exerted weak selection pressures on invertebrate assemblages in New Zealand lakes and ponds (Wissinger et al. 2006, Greig et al. 2013). I did find that some invertebrates using flexible or inflexible life-history strategies could experience high mortality due to predatory fish, but each generalist species had behaviours that likely mitigated predation risks in the field (Chapters Two and Three). Specifically, permanent-pond *X. zealandica* reduced their movements and spent more time in refuges in the presence of fish, while *S. arguta* were more likely to be found in permanent ponds with shallow margins that might provide refuge from fish (Chapters Two and Three). Thus, through flexible anti-predator behaviours and occupying habitat refugia these invertebrates may reduce risks of occupying permanent ponds, and reduce selection pressure for specialised predator-avoidance traits (e.g, morphology and behaviour) typically observed in stable environments in North America and Europe (McPeek 1990, Johansson 2000, Wissinger et al. 2003).

Overall then, New Zealand's young geological history combined with a mild temperate climate with oceanically-driven unpredictable rainfall likely allows generalist species to persist across a disturbance gradient by using life-history strategies which weaken the contrasting abiotic and biotic risks. Nevertheless, disturbances that are greater in magnitude, less predictable, or more frequent could result in higher selection pressures, forcing invertebrates to make greater trade-offs across habitat-disturbance gradients. Thus, how species sustain populations across unpredictable disturbance gradients could influence their

vulnerability to global changes including climate warming and changing land-use, depending on the constraints associated with their life-history strategies.

Will constraints on generalist life-history strategies result in uneven climate-change responses?

At the global scale, warming and shifts in precipitation patterns are altering the distributions of a wide range of species, favouring species that are more resilient to changing environments or those who are able to disperse to more beneficial habitats (Arribas et al. 2012a). Increasingly unpredictable disturbances resulting from climate change are more likely to favour species that have broad realised niches with generalist life-history strategies, and dispersal patterns that permit them to exploit a range of environments (Clavel et al. 2011, Lurgi et al. 2012, Ponge 2013). Generalist species with particular life-history strategies might do better in unpredictable conditions than some specialists, but the constraints of their specific life-history strategies could result in uneven responses to climate change. I found two aquatic invertebrates species persisted across a wide pond-permanence gradient, but used slightly different life-history strategies, which will likely affect their vulnerability to changes in the predictability of disturbance (Chapter Two). My results suggest generalist life-history strategies based on flexible versus inflexible strategies might fare differently with further inter- and intra-annual variation in drying disturbance under future global climate changes.

Depending on how climate change might alter the magnitude, predictability and frequency of environmental disturbances, it could reinforce generalist life-history strategies or favour adaptation and specialisation across habitat disturbance gradients (Bourne et al. 2014). Currently, hydrologic regimes of many of New Zealand's freshwater habitats depend on highly unpredictable precipitation patterns (Winterbourn et al. 1981, Clausen and Biggs 1997,

Wissinger et al. 2009). Over the last 35 years there has been a drying trend during the austral summer across most of New Zealand, and greater rainfall variability has been projected with future warming (Griffiths 2007, Ummenhofer et al. 2009). Associated with these higher temperatures, extreme weather events, including both floods and droughts, are predicted to be twice as frequent over the next century (Christensen et al. 2013). These trends toward higher temperatures, along with more frequent and severe weather events, will likely result in less predictable hydrology of aquatic habitats. Increases in both unpredictability and magnitude of disturbances have the potential to influence distributions of invertebrates with generalist life-history strategies.

I tested how the combination of increased temperature and variability of precipitation under climate change scenarios might influence distributions of the generalist invertebrates studied. I used down-scaled climate projections for the Canterbury high-country and adjusted the water temperature and water depth data collected during the course of my study (2010-2013) to determine the potential number of available growth days for each species, as calculated in Chapter Two, under future climate scenarios. Based on the developmental thresholds for *X. zealandica* (135 days) and *S. arguta* (61 days) I estimated the number of temporary ponds likely to support each species' life-history strategy. This approach gives insight into how generalist distributions in temporary ponds might be influenced by changes in precipitation by using relatively uncomplicated hydrological assumptions.

The down-scaled temperature and percent precipitation projections for 2040 and 2080 were based on twelve global climate models that were re-scaled to match 2007 IPCC global warming ranges for six emission scenarios (Ministry for the Environment, New Zealand, 2008). Using the mean annual predictions (2040: +0.9^{o}C, -1% precipitation; 2080: +2^{o}C, -2%) along with the potential lower (2040: +0.2^{o}C, -10%; 2080: +0.7^{o}C, -14%) and upper

ranges (2040: +1.9^{o}C, +9%; 2080: +5^{o}C, +16%), I was able to adjust the continuous water logger data for the number of days above each species' growth threshold (*X. zealandica*: 8^{o}C, *S. arguta*: 10^{o}C), and the proportion of days each temporary pond held water.

From these climate estimates I found there were minor increases in the proportion of temporary ponds available for both species using the mean annual temperature and precipitation predictions (Figure 5.4). This small increase is likely due to warmer winters that might permit some more growth than currently occurs. However, from the upper and lower forecasts, there were greater variations in the proportion of ponds available for *X. zealandica* than *S. arguta* (Figure 5.4A and B). Because *S. arguta* require fewer days to complete development they are likely to be influenced less strongly by hydrologic changes than *X. zealandica,* which require longer wet periods to complete nymph development. Overall, this suggests that *S. arguta*'s less flexible life-history strategy might gain a selective advantage in increasingly unpredictable temporary ponds (Figure 5.5B). In contrast, *X. zealandica* might experience boom and bust populations associated with greater inter-annual oscillations in temporary pond availability if precipitation is highly variable with frequent wet and dry years, as has been forecasted for New Zealand (Figure 5.5A).

With inter-annual variability in precipitation increasing there are also predictions of more frequent severe droughts in New Zealand's South Island, rising from one in twenty years to every five years (Mullan et al. 2005). Thus, during droughts temporary ponds may dry, along with many semi-permanent ponds, leaving only permanent ponds as potential population refuges. As a result, increasingly variable climate conditions could cause greater source-sink dynamics across the pond-permanence gradient, with recruitment from permanent ponds becoming more critical. Drought disturbance can greatly impact freshwater ecosystems, yet there is evidence that New Zealand stream invertebrate communities might be resilient to

drought conditions through recolonisation from neighbouring habitats and adaptations to drying disturbances (Caruso 2002, Arscott et al. 2010, Storey and Quinn 2011).

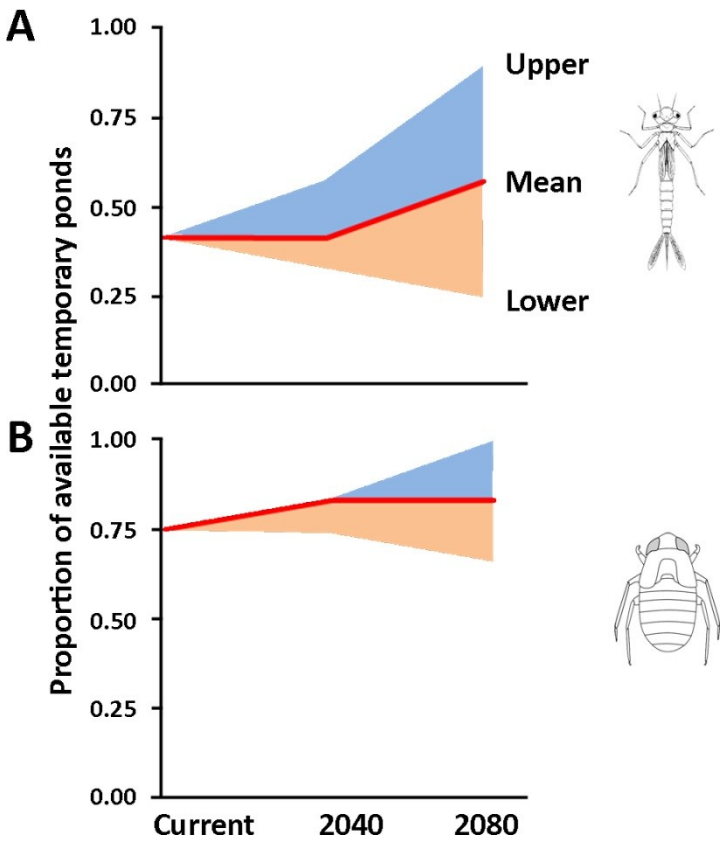

Figure 5.4. Estimation of the proportion of temporary ponds available for two invertebrates, *X. zealandica* (A) and *S. arguta* (B) under three climate scenarios associated with inter-annual variability in temperature and precipitation with mean (red line), upper (wetter; blue), and lower (drier; orange) predictions. These species use alternative generalist life-history strategies, to exploit a range of environments across a disturbance gradient of pond-drying. *Xanthocnemis zealandica* had a flexible, but longer developmental strategy; whereas *S. arguta* had inflexible rapid development across the disturbance gradient. Overall, *X. zealandica* had longer developmental periods (≥ 135 growth days) than *S. arguta* (≥ 61 growth days) which might result in the generalist with an inflexible life-history strategy experiencing less variability in their ability to exploit temporary ponds under climate change. These scenarios were based on downscaled climate predictions for the 2040 and 2080 changes in Canterbury high-country temperature and precipitation (Ministry for the Environment, New Zealand, 2008), and developmental thresholds calculated for each species in Chapter Two.

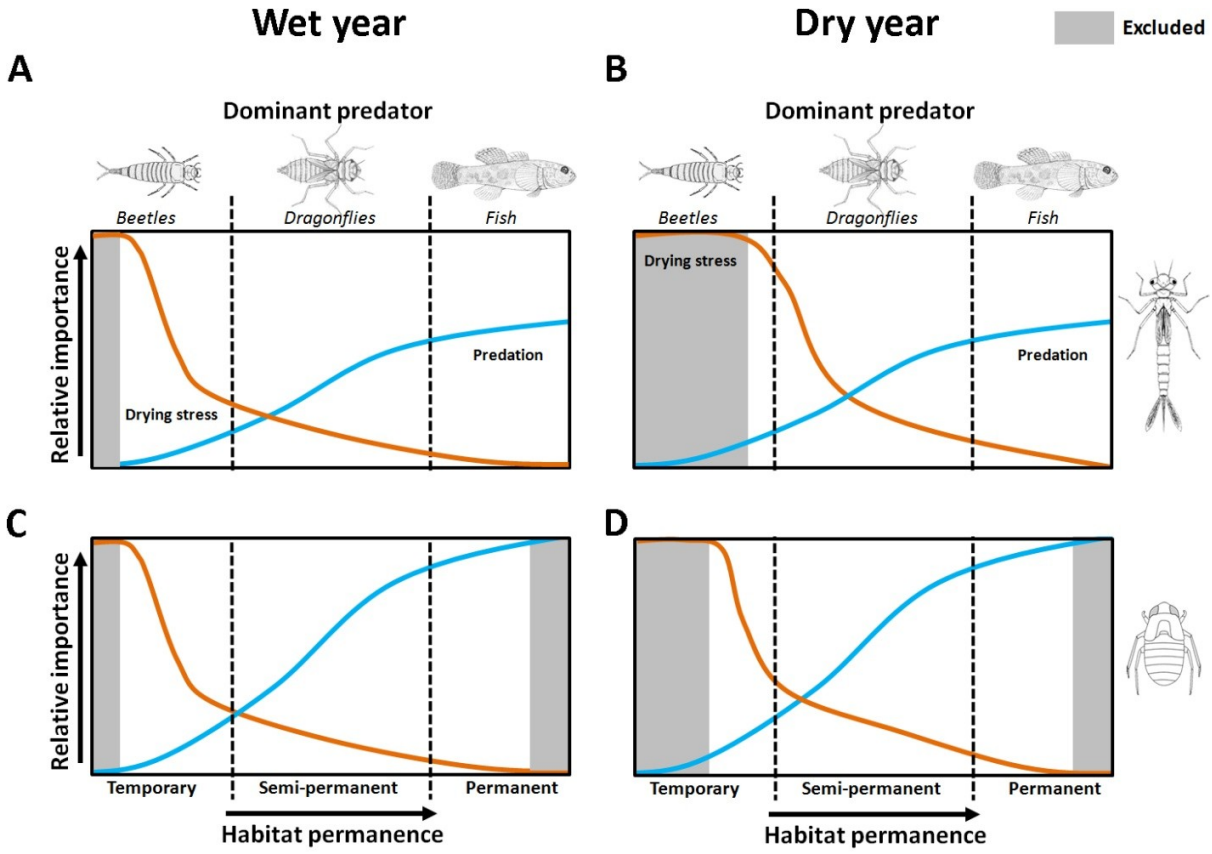

Figure 5.5. Potential influences of altered climate changes on the distribution patterns of *X. zealandica* (A) and *S. arguta* (B) across the modified Wellborn et al. (1996) predator-permanence gradient. Grey areas indicate portions of the permanence gradient where these species might be excluded. Due to longer juvenile development requirements (≥ 135 growth days), *X. zealandica* might permit them to exploit most ponds during wetter years (A) while they might be excluded from most temporary ponds during harsher dry-years (B) across the pond permanence-gradient. Due to *S. arguta*'s rapid juvenile development (≥ 61 growth days) they will likely experience less variability in their distributions during wet- and dry-years (C - D). Distributions of both generalist species in more stable habitats are unlikely to change due to climate change.

Although many aquatic invertebrate populations might be resilient to drought conditions, there can be long-term impacts from high magnitude and long duration disturbances, such as community shifts and population bottlenecks that reduce genetic diversity (Humphries and Baldwin 2003, Bogan et al. 2011, Huey et al. 2011, Shama et al. 2011). During the course of this study I found population turnover in some temporary ponds, but there was no evidence of

contemporary population bottlenecks from the microsatellite analyses (Chapter Four). This suggests that the close proximity of permanent-pond population sources and strong dispersal capacity resulted in genetic mixing that limited the impact of unpredictable drying disturbances in portions of the pond-complex metapopulations. Therefore, dispersal among permanent and temporary habitats will likely be critical to sustain regional populations under further unpredictable and frequent disturbances, and may be more important than an ability to tolerate changing environmental conditions (Chester and Robson 2011, Huey et al. 2011, Arribas et al. 2012b). Because gene flow can reduce the likelihood of population bottlenecks, it is paramount to protect and manage a wide range of habitats across disturbance gradients to maintain robust metapopulations under climate change.

Overall, unpredictable disturbances likely ameliorate life-history trade-offs, favouring species with wide niches, but more frequent and longer duration disturbances from climate changes could result in uneven generalist responses and influence speciation. Around the world unpredictable disturbances resulting from global changes (e.g., climate change, biotic invasions, anthropogenic impacts) are driving a trend toward community homogenisation, favouring generalist species that can exploit a wide range of habitats (Clavel et al. 2011, Lurgi et al. 2012). However, as I found, generalist species can have different life-history strategies to exploit habitats with unpredictable disturbances, which could influence their response to additional disturbances. Therefore, whilst generalist species might have an advantage in habitats experiencing frequent unpredictable disturbances, shifts toward more frequent and longer duration disturbances may reinforce or jeopardise different generalist life-history strategies. Further disturbances might favour life-history strategies with early colonization and rapid development (Sim et al. 2013) over more flexible strategies, which might suffer greater population fluctuations. Moreover, climate change might alter how various generalist species experience life-history trade-offs across disturbance gradients. For

example, if generalist species experience higher selection pressures in intermittent habitats there may be greater advantages for adaptations to avoid or tolerate harsh disturbances, potentially driving speciation. Thus, unpredictable ecosystems dominated by generalist species may also be vulnerable to climate shifts and result in communities dominated by species with highly tolerant or transient life-histories. Therefore, understanding the life-history strategies that permit generalists to counter selection pressures across habitat disturbance gradients provides insights on how further unpredictable disturbances might influence population persistence, community ecology and species evolution under climate change.

Plate 9. Sunset over a temporary pond, part of the Ō Tū Wharekai wetland system, within the Hakatere Conservation Area.

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Plate 10. Winter sampling at Lake Donne, part of the Ō Tū Wharekai wetland system, within the Hakatere Conservation Area.

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